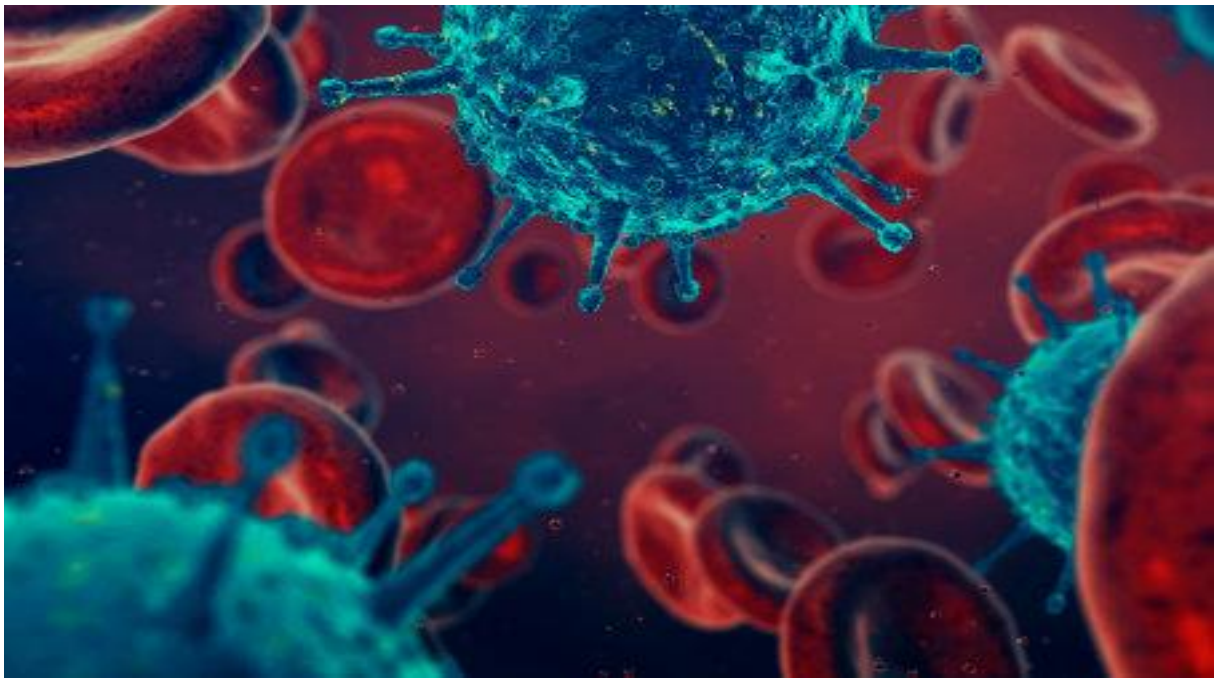
BASIC IMMUNOLOGY FOR RHEUMATOLOGY



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Introduction to the immune system

- Immunity was typically defined as resistance to, protection from or defense against diseases mainly infectious. Additionally the immune responses are directed against tumors and transplants; also participate in the clearance of dead cells and initiation of tissue repair.

- The immune system "like any system" includes:
  - *Organs*
  - *Specialized cells "immune cells"*
  - *Molecules*

- Reactions mediated by those cells and molecules are called the ***immune responses.***

Organs

- Primary lymphoid organs
  - ✓ Bone marrow
  - ✓ Thymus

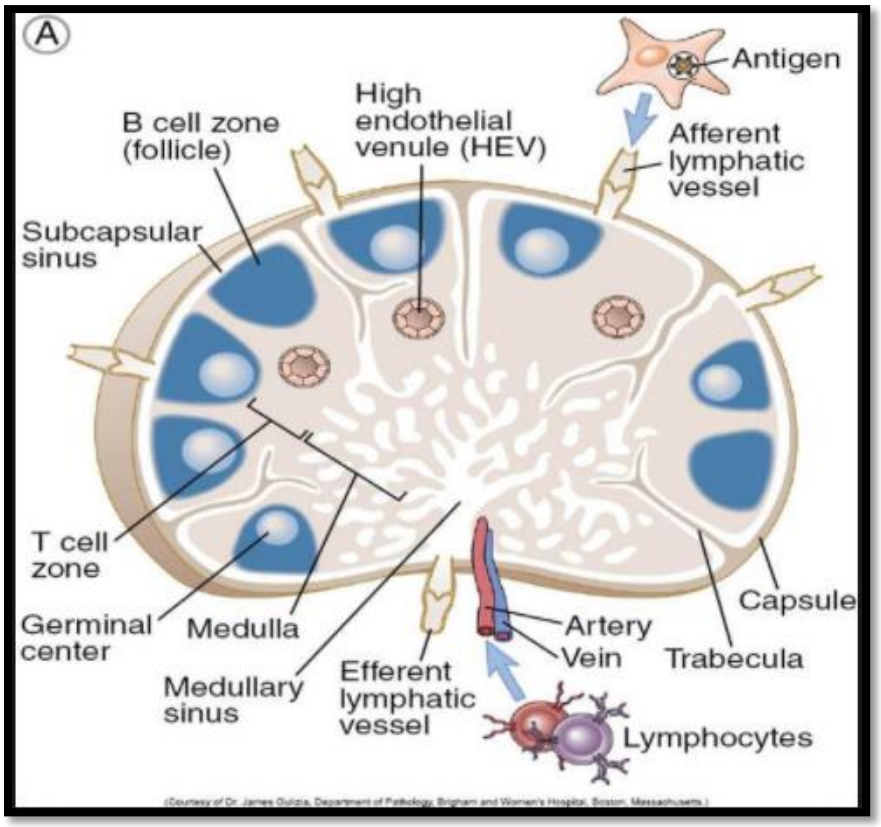
- Secondary lymphoid organs
  - ✓ Lymph nodes
  - ✓ Spleen
  - ✓ Cutaneous lymphoid tissue
  - ✓ Gut associated lymphoid tissue (GALT)
  - ✓ Mucosa associated lymphoid tissue (MALT)

- Primary lymphoid organs also known as "***generative or central***" are sites of development of immune cells.

- Secondary lymphoid organs also known as "***peripheral***" are considered the residence for lymphoid cells and the "traps" for pathogens in different tissues. They are organized to optimize interactions of antigens, antigen presenting cells (APs) and lymphocytes in a way that promotes the development of adaptive immune responses.

- In the lymph nodes the B lymphocytes reside in the follicles as they express a chemokine receptor ($CXCR_5$) specific to a chemokine produced in this region ($CXCL_{13}$). T lymphocytes reside in the paracortex as they express a chemokine receptor (CCR_7) specific to chemokines (CCL_{19}& CCL_{21}) produced in this region.

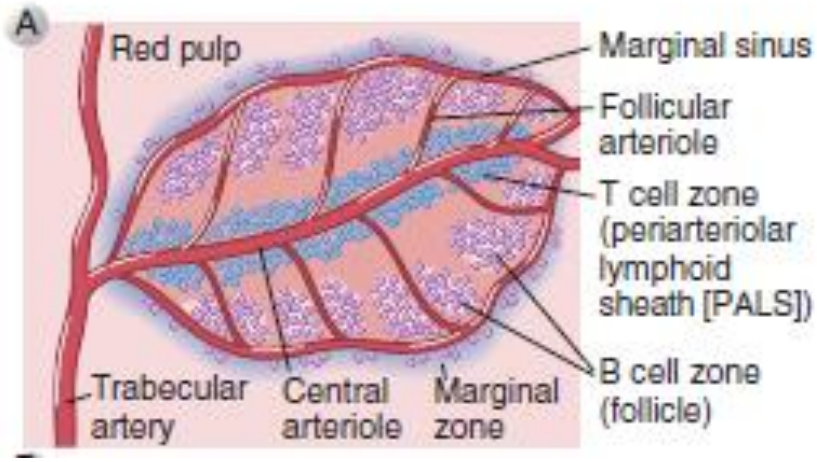
- Lymph is the fluid that constantly leaks out of blood vessels in all epithelia and connective tissues and most parenchymal organs. Lymph is drained by lymphatic vessels to lymph nodes and eventually back onto blood circulation. Therefore the lymph contains a mixture of substances 'among them the pathogen in a soluble form' absorbed from epithelia and tissues.



- The spleen is a highly vascularized organ whose major functions are:
  1. Removal of aging and damaged blood cells and particles 'such as immune complexes and opsonized microbes' from the circulation
  2. Initiating adaptive immune response to blood borne antigens.

- Anatomically and functionally divided into:

1. ***Red pulp*** which is composed mainly of blood filled vascular sinusoids.
2. ***White pulp*** rich in lymphocytes, its architecture is analogous to the organization of lymph nodes.
3. ***Marginal zone***: A region of specialized cells "specialized macrophages and marginal B cells" surrounding the marginal sinus forms the boundary between the red and the white pulp.



Specialized cells

- Cells of the immune system develop from a common progenitor, the hematopoietic Stem cell "HSC" in the bone marrow which give rise to the:
  - ✓ ***Myeloid lineage***: Neutrophils, Eosinophils, Basophils, Monocytes and Dendritic cells (DCs).
  - ✓ ***Lymphoid lineage***: T & B lymphocytes, Natural killer (NK) cells and DCS.

- They are present as:
  - Circulating cells in the blood and lymph
  - Anatomically defined collections in lymphoid organs
  - Scattered cells in virtually all tissues

The *homing* of these cells at different tissues and their ability to *exchange* among blood, lymph and tissues are of critical importance for the generation of immune responses.

Molecules

They are so many, among them:

- Cluster of differentiation (CDs)
- Cytokines
- Chemokines
- Complement proteins
- Adhesion molecules
- Signaling molecules
- Transcription factors
- Antigen recognition receptors
- Human leucocyte antigen (HLA) molecules
- Acute phase proteins

- Functionally the immune system is classified into:
  - ✓ **Innate immune system**
  - ✓ **Adaptive immune system**

- Each system has its components and responses. They are not acting separately indeed many *bidirectional* connections are present integrate and complement the immune reactions and responses.

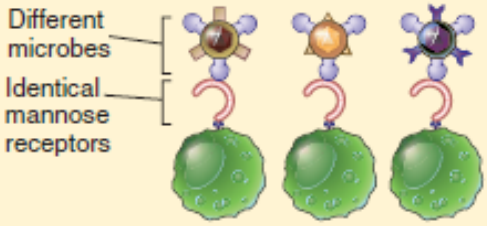
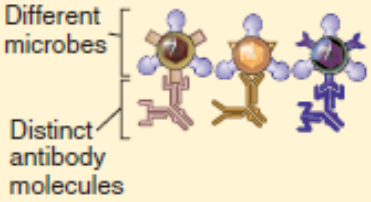
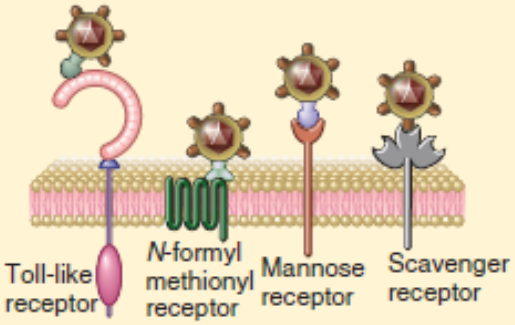
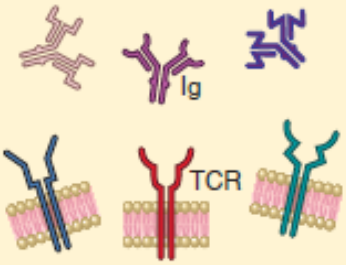
- Innate immune response includes:
  - ***Physicochemical reactions***
  - ***Cellular response:*** Phagocytosis, NK cell activity……
  - ***Humoral response***: Offered by complement proteins and other acute phase proteins

- Adaptive immune response includes:
  - ***Humoral immune response***: Antibodies secreted by plasma cells, complement proteins.
  - ***Cell mediated immune response*** by T-helper and T-cytotoxic cells with enhancement of phagocytosis or direct killing of host cells respectively.

- The main reactions are:
  - Inflammation
  - Phagocytosis
  - Antiviral effect

- The innate and adaptive immune systems differ in many aspects:
  - ✓ Components
  - ✓ The way each system recognizes the antigen
  - ✓ The time of interaction
  - ✓ Specialization
  - ✓ The homeostatic mechanisms
  - ✓ However one of the most important differences is ***memory***. The innate system is said to have no or little memory i.e. reacts in the same way with each exposure to antigen.

TABLE 4.1 Specificity of Innate and Adaptive Immunity

	Innate Immunity	Adaptive Immunity
Specificity	For structures shared by classes of microbes (pathogen-associated molecular patterns) 	For structural detail of microbial molecules (antigens); may recognize nonmicrobial antigens 
Number of microbial molecules recognized	About 1000 molecular patterns (estimated)	>10^7 antigens
Receptors	Encoded in germline; limited diversity (pattern recognition receptors) 	Encoded by genes produced by somatic recombination of gene segments; greater diversity 
Number and types of receptors	<100 different types of invariant receptors	Only 2 types of receptors (Ig and TCR), with millions of variations of each
Distribution of receptors	Nonclonal: Identical receptors on all cells of the same lineage	Clonal: clones of lymphocytes with distinct specificities express different receptors
Genes encoding receptors	Germline encoded, in all cells	Formed by somatic recombination of gene segments only in B and T cells
Discrimination of self and nonself	Yes; healthy host cells are not recognized or they may express molecules that prevent innate immune reactions	Yes; based on elimination or inactivation of self-reactive lymphocytes; may be imperfect (giving rise to autoimmunity)

Ig, Immunoglobulin; *TCR*, T cell antigen receptor.

Innate immunity

- Also called *natural or native*, considered as the first line of defense against infection.
- Always present, ready to recognize and eliminate or control infections. Doesn't require prior exposure to the microbe.
- Cells of the innate system respond to *allergens*.
- The innate immune system specifically recognizes and reacts against microbes through cell associated *pathogen recognition receptor (PRR)* and soluble receptors that recognize structure shared by various classes of microbes; *pathogen associated molecular patterns (PAMP).*
- The innate immune system can also be triggered by molecules released from damaged, stressed or necrotic cells; *damage associated molecular pattern (DAMP),* hence responsible for clearance of dead tissue and initiation of tissue repair.
- Innate immune system reacts in essentially the same way with repeated infections i.e. *no or little memory*.
- Cells of the innate system activate and modulate the adaptive immune response.

Components & effector molecules of the innate immune system:

I. Epithelial barriers
II. Circulating & tissue cells
III. Circulating proteins
IV. Cytokines

I. Epithelial barriers

- Skin and mucosa of gastrointestinal (GIT), respiratory and genitourinary tract represent the major interfaces between the body and the external environment.
- Continuous epithelia may physically interface with the entry of microbes.
- Epithelial cells also produce peptides with antimicrobial activities "natural antibiotis", defensins and cathelicidins.
- Intraepithelial T lymphocytes &ɣ/δ T cells that belong to T cell lineage with limited specificities of their Antigen recognition receptors. They may recognize microbial lipids & provides fast response against microbes that enter through epithelia.
- Other physical & chemical features that aid in defense: cilia, tears, PH acidity.

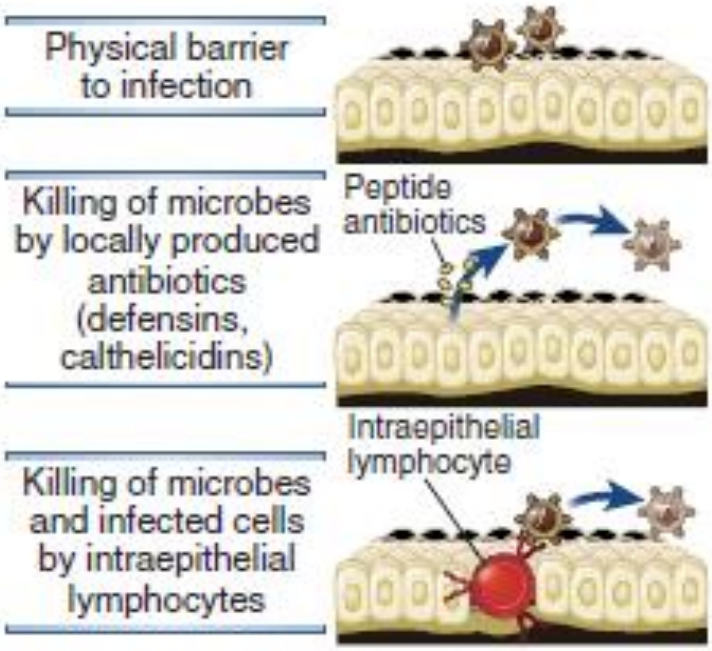


FIGURE 4.7 Epithelial barriers. Epithelia at the portals of entry of microbes provide physical barriers, produce antimicrobial substances, and harbor intraepithelial lymphocytes that are believed to kill microbes and infected cells.

II. Circulating & tissue cells

- Monocytes/macrophages
- Neutrophils
- Dendritic cells
- Eosinophils
- Basophils
- Mast cells
- NK cells and other Innate lymphoid cells (ILCs)

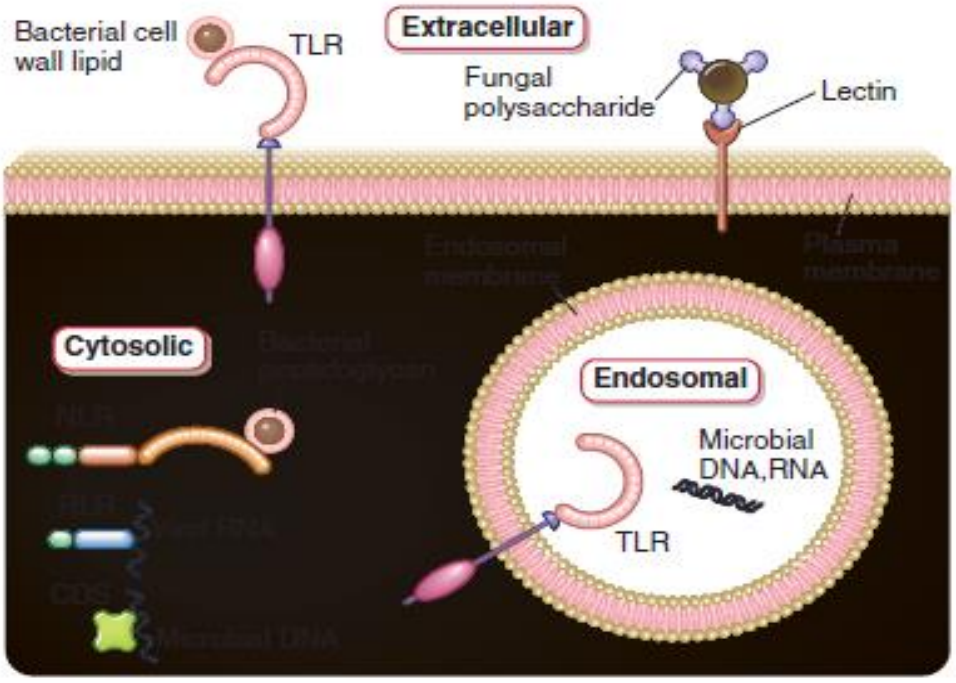
Other lymphocyte subsets that behave like innate cells: Invariant NK-T cells (iNKT), ɣ/δ T cell, intraepithelial T cell, B1 cell, marginal zone B cell.

- The way the innate system recognizes pathogen or foreign antigen differs from that of the adaptive system. Recognition occurs via *PRRs*.

- PRRs can be divided into 2 broad groups:

1. **Trans-membrane and intracellular proteins** (signal-transducing receptors)
2. **Secreted and circulating proteins & peptides**- These proteins & peptides mediate:
   - ✓ Direct microbial killing
   - ✓ Act as helper for transmembrane receptors
   - ✓ Act as opsonins for phagocytosis by innate & adaptive immune cells

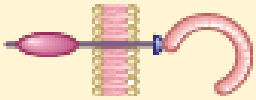
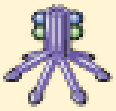
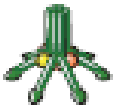
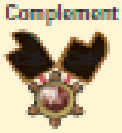
- PRRs recognize structures shared by classes of microbes called PAMP e.g. endotoxin "LPS" present in the cell wall of many bacterial species.
- PRRs also recognize molecules expressed damaged cells, DAMP.
- PRRs are encoded in germline & have limited diversity than those of lymphocyte antigen recognition receptors.
- The distribution of cell associated PRRs is non-clonal i.e. identical receptors on all cells of the same lineage.
- PRRs are expressed in different cellular compartments where microbes may be located (extracellular, endosomal or cytosolic).



- How do PRRs identify only microbes and not self (host tissues)?
  - ✓ Because PAMP structures have certain features in common:
  1. They are produced only by microbes and not their hosts.
  2. They are typically invariant structures shared by the entire class of pathogens
  3. They are usually fundamental to the integrity, survival and pathogenicity of the microbe. The microbe cannot mutate its PAMP to avoid host's defense system and still survive.

- Examples of PAMPs:
  1. Bacterial endotoxin is a prototypical PAMP (lipopolysaccharide component of the outer membrane of all gram -ve bacteria)
  2. Other bacterial membrane components such as peptidoglycan, lipoteichoic acid & mannans.
  3. Unmethylated microbial DNA
  4. Double stranded RNA of viral origin
  5. Glucans, polysaccharides or proteins common to microbes but not animals or humans.

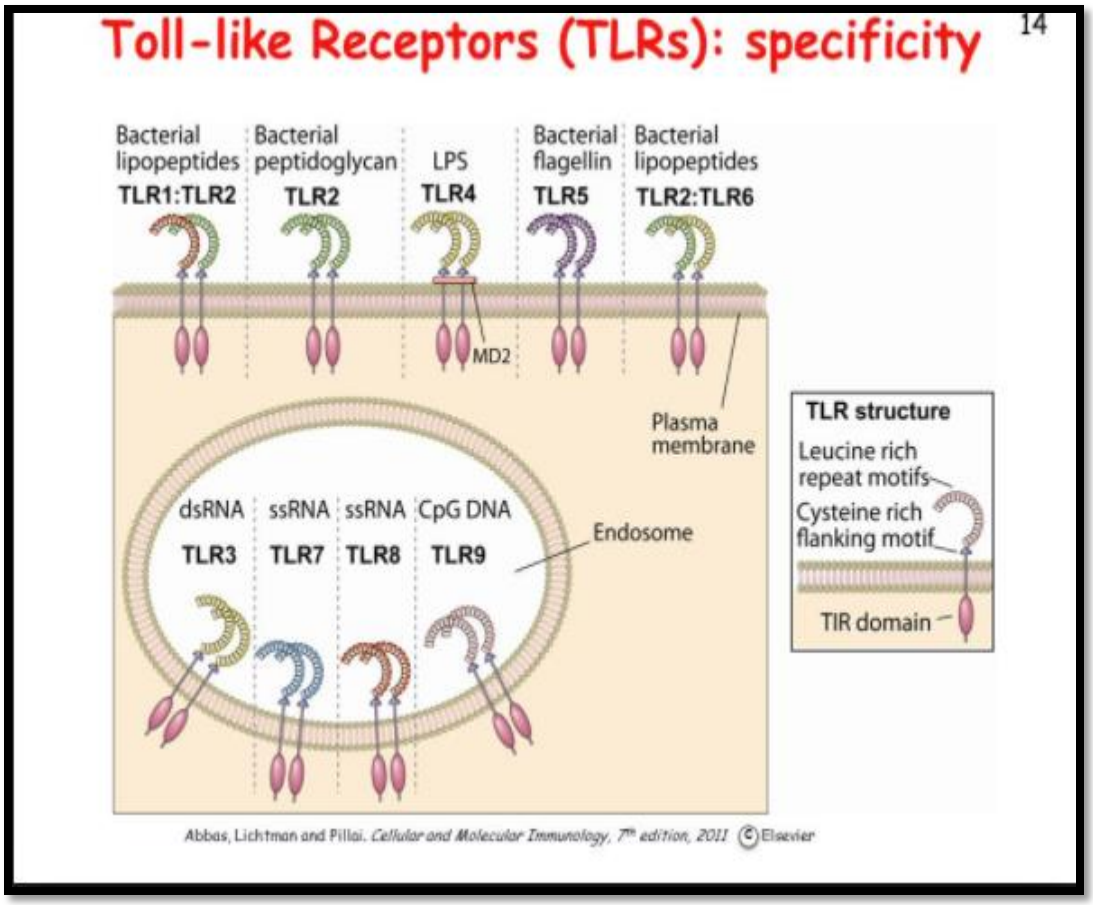
Families of cell associated PRRs:

Pattern Recognition Receptors	Location	Specific Examples	Ligands (PAMPs or DAMPs)
Cell-Associated			
TLRs 	Plasma membrane and endosomal membranes of DCs, phagocytes, B cells, endothelial cells, and many other cell types	TLRs 1–9	Various microbial molecules including bacterial LPS and peptidoglycans; viral nucleic acids
NLRs 	Cytosol of phagocytes, epithelial cells, and other cells	NOD1/2 NLRP family (inflammasomes)	Bacterial cell wall peptidoglycans Intracellular crystals (urate, silica); changes in cytosolic ATP and ion concentrations; lysosomal damage
RLRs 	Cytosol of phagocytes and other cells	RIG-1, MDA-5	Viral RNA
CDSs 	Cytosol of many cell types	AIM2; STING-associated CDSs	Bacterial and viral DNA
CLRs 	Plasma membranes of phagocytes	Mannose receptor DC-sign Dectin-1, Dectin-2	Microbial surface carbohydrates with terminal mannose and fructose Glucans present in fungal and bacterial cell walls
Scavenger receptors 	Plasma membranes of phagocytes	CD36	Microbial diacylglycerides
N-Formyl met-leu-phe receptors 	Plasma membranes of phagocytes	FPR and FPRL1	Peptides containing *N*-formylmethionyl residues
Soluble			
Pentraxins 	Plasma	C-reactive protein	Microbial phosphorylcholine and phosphatidylethanolamine
Collectins 	Plasma Alveoli	Mannose-binding lectin Surfactant proteins SP-A and SP-D	Carbohydrates with terminal mannose and fructose Various microbial structures
Ficolins 	Plasma	Ficolin	*N*-acetylglucosamine and lipoteichoic acid components of the cell walls of gram-positive bacteria
Complement 	Plasma	Various complement proteins	Microbial surfaces

AIM2, Absent in melanoma; *CDSs*, cytosolic DNA sensors; *CLRs*, C-type lectin–like receptors; *DAMP*, damage-associated molecular pattern; *DC*, dendritic cells; *MDA*, melanoma differentiation-associated gene; *NLRs*, NOD-like receptors; *NOD*, nucleotide oligomerization domain; *PAMP*, pathogen-associated molecular pattern; *RLRs*, RIG-like receptors; *SP-D*, surfactant protein D; *STING*, stimulator of IFN genes; *TLRs*, toll-like receptors.

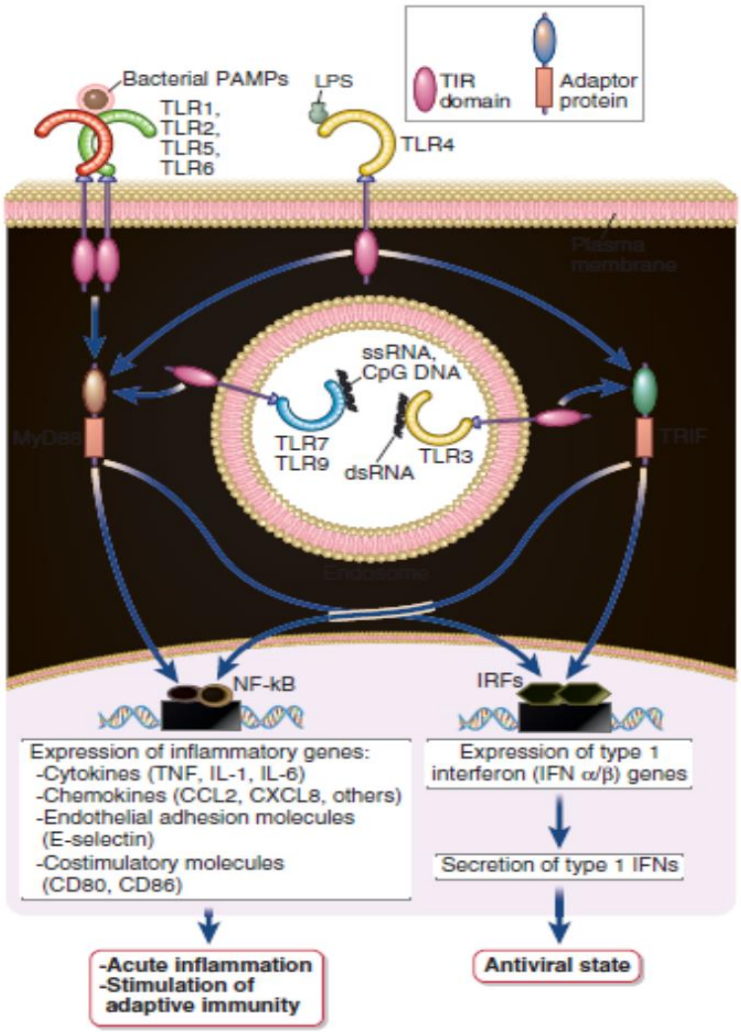
Families of cell associated PRRs:

- ***Toll like receptors (TLRs)***: homologous to a drosophila protein called Toll. Some are extracellular, others are endosomal.
  - ✓ TLR_2: recognize several bacterial lipoglycan.
  - ✓ TLR_3, TLR_7 &TLR_8: recognize viral nucleic acids.
  - ✓ TLR_4: recognize bacterial lipopolysaccharide.
  - ✓ TLR_5: recognize bacterial flagellin.



Engagement of TLRs leads to activation of many transcription factors:

1. NFκB: Expression stimulates many pro-inflammatory genes including genes of IL-1, IL-6 & TNF-α in addition to adhesion molecules and co-stimulators.
2. IRFs "Interferon regulatory factors": expression of type 1 interferons.



- ***NOD like receptors (NLRs)***: cytosolic receptors that sense DAMP & PAMP in the cytoplasm.
  - ✓ NOD= Nuleotide oligomerization domain
  - ✓ NOD like receptors are a family of more than 20 cytosolic receptors they recruit other proteins to form *signaling complexes.*

There are 3 subfamilies named after the domain "part of the receptor" that recruit the protein needed to form the signaling complexes:

1. CARD subfamily "caspase recruitment domain"
   - ✓ NOD1 & NOD2 are members NOD signalosome.
   - ✓ Loss of function mutation of NOD2 is associated with crohn's disease.
2. Pyrin subfamily: $NLRP_3$, $NLRP_5$ & $NLRP_4$ are members also called **cryoprin**. Their signaling complexes are called inflammasomes.
3. BIR subfamily

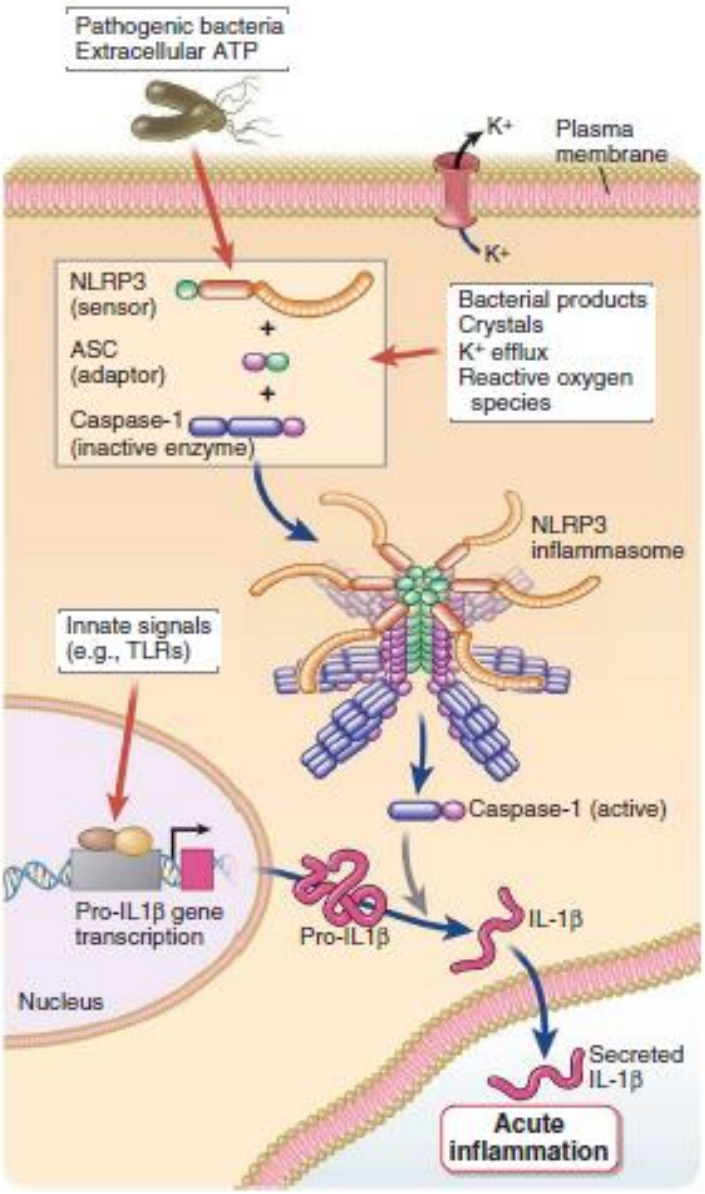
Inflammasome

- The best studied is that formed by:
  1. *$NLRP_3$ oligomer*
  2. *Adaptor protein (ASC)*
  3. *Inactive caspase*

- It is activated when sensing:
  - Bacterial products
  - Crystals: Monosodium urate crystal (MSU), calcium pyrophosphate crystals, cholesterol, lipids, asbestos, silica
  - K^+ efflux
  - Reactive O2 species
  - B-Amyloid in Alzheimer's disease
  - Extracellular ATP released from dead cells in the cytoplasm of the responding cell

- Sequences: When the sensory part of the inflammasome senses any of the previous cytoplasmic stimuli → activation of caspase 1→ cleavage of the precursor form of IL-1β & IL-18→ active IL1β & IL-18→ *Acute inflammation.*

- Inflammasome activation of caspase 1 may also lead to a form of programmed cell death (PCD) called "***Pyroptosis".***



- Dysregulated activation of the inflammasome due to autosomal gain-of-function mutation in one or another of its component proteins→ Excessive IL-1 production→ Recurrent attacks of fever & localized inflammation most commonly in joints & GIT which are called "***Cryoprin associated periodic syndromes (CAPS)"***

- Mutation affecting constituent of the inflammasome→ ***"Familial autoinflammatory syndrome (FMF)"***

- ***RIG like receptors***
  - ✓ Are also cytosolic receptors that recognize viral RNA→ Production of type 1 interferon via activation of the transcription factors ***" IFR_3, IFR_7 & NFκB "***

  - Members:

1. RIG-1"Retinoic acid inducible gene"
2. MDA5 "Melanoma differentiation associated gene 5"

- ***Cytosolic DNA sensors "CDS_5"***
  - ✓ Detect cytosolic DNA
  - ✓ When stimulated they activate signaling pathways that initiate:
    1. Antimicrobial responses including type 1 interferon
    2. Autophagy

  - Members:

1. STING "Stimulator of IFN genes"
   - ✓ STING pathway leads to:
     a. Activation of IRF3 → Type 1 interferon
     b. Autophagy
2. RNA polymerase 3→ Activates RIG pathway→ Type 1 interferon expression.
3. AIM2 "Absent in melanoma-2"→Forms caspase 1 containing inflammasome→IL-1 & IL-18 activation.

- ***Receptors for carbohydrates***
  - ✓ These receptors recognize carbohydrates on the surface of microbes
    a. Facilitate the phagocytosis of microbes.
    b. Secretion of cytokines that promote subsequent adaptive IR.

  - Members:

1. Mannose receptors: One of the C-type lectin (CD206) recognizes terminal sugar on microbial surface and act as opsonin.
2. Detectins: Detectin 1 & 2 "Dendritic cell associated C-type lectin"
   - ✓ Bind fungal glycan signaling events that facilitate cytokines & proteins from dendritic cell that promote adaptive IR; facilitate differentiation of CD4+ to Th17.

- ***Scavenger receptors***
  - ✓ Family of cell surface receptors expressed on macrophages originally defined as receptors that mediate the uptake of oxidized oracetylated low density lipoprotein (LDL) members: SR-A & CD36
  - ✓ They also mediate the phagocytosis of a variety of microbes through recognizing a variety of PAMPs including LPS, lipoteichoic acid, β-glycan & others

- ***Formyl-peptide receptors***
  - ✓ The formyl peptide receptor-1 (FPR-1) expressed on leucocytes recognizes bacterial peptides containing N-Formyl methionyl reidues which is considered one of the most potent chemoattractants→ Stimulate directed movement of leucocytes.

Soluble PRRs:

- ***Pentraxins***
  - ✓ It recognizes microbial phosphorylcholine and phosphatidylethanolamine.
  - ✓ They also bind to C1q and activate the complement pathway

  - Members
  1. CRP is the most famous member of this family
  2. Serum amyloid P "SAP"
  3. Long pentraxin"PTX_3"

- ***Collectins***

✓ Recognize carbohydrates

  - Members:
  1. Mannose binding lectin: opsonin and iniates the MBL complement pathway.
  2. Surfactant proteins "SP-A & SP-D": They function to maintain the ability of lungs to expand.

- ***Ficollins***

✓ Ficollins recognize lipoteichoic acids in cell wall of gram +ve bacteria→ Opsonization

- ***Complement proteins***

Neutrophils

- Also called polymorphnuclear leukocytes, the most abundant population of WBCs. The nucleus is segmented to 3 or 5 lobules containing cytoplasmic granules which don't stain with either acidic or basic dyes.

- Granules of neutrophils are either:
  1. Specific- contain:
     a. Lysozyme
     b. Collagenase
     c. Elastase
  2. Azurophilic "lysosomes"- contain:
     Enzymes and microbicidal substances including defensins & cathilithidin

- Considered the first cell type to respond to infection
- Their number increases from bone marrow in response to colony stimulating factors (CSFs) secreted by many cell types in response to infection.
- They ingest microbes in the circulation & in tissues after extravascular migration and die in few hours. Accordingly, they do not provide prolonged defense.
- They are also recruited to sites of tissue damage where they initiate clearance of cell debris.
- **Neutrophilic extracellular traps (NETs):** Neutrophils extrude their nuclear & granular contents forming a network of histones and other components with microbicidal activities. These NETs trap bacteria & fungi and kill the organisms in a process called **NETosis.**

Macrophages/ Monocytes

- Less abundant than neutrophils.
- No: 500-1000 cells/ml
- Structure: They have a bean shaped nucleus & finely granular cytoplasm.
- They ingest microbes in the blood & tissue but unlike neutrophils, they live for longer periods.
- Specialized macrophages are present in many tissues; e.g. kupffer cells in the liver, alveolar macrophages in the lung, microglial cells in the brain.

- They are heterogeneous;
  1. Classic monocytes are $CD14^{+}$ $CD16^{-}$
  2. Non classical are $CD14^{+}$ $CD16^{++}$
  3. Intermediate are $CD14^{++}$ CD 16^{+}

- Their major function is to ***ingest and kill microbes***. Other functions include:
  1. Cleaning up process after infection& sterile tissue injury: They ingest dead tissue cells & dying neutrophils.
  2. Engulf apoptotic cells before releasing their content.
  3. Secrete several cytokines.
  4. Serve as Antigen presenting cells (APCs).
  5. Promote the repair of damaged tissues by stimulating new blood vessel growth and collagen synthesis.

- There are 2 pathways of activation "classical & alternative" according to the nature of stimuli that activate macrophages:

1. *Classical activation pathway*-induced by:

a. Innate immune signals e.g. microbial stimulation of TLRs.
b. INF-ɣ from NK or activated T cells.

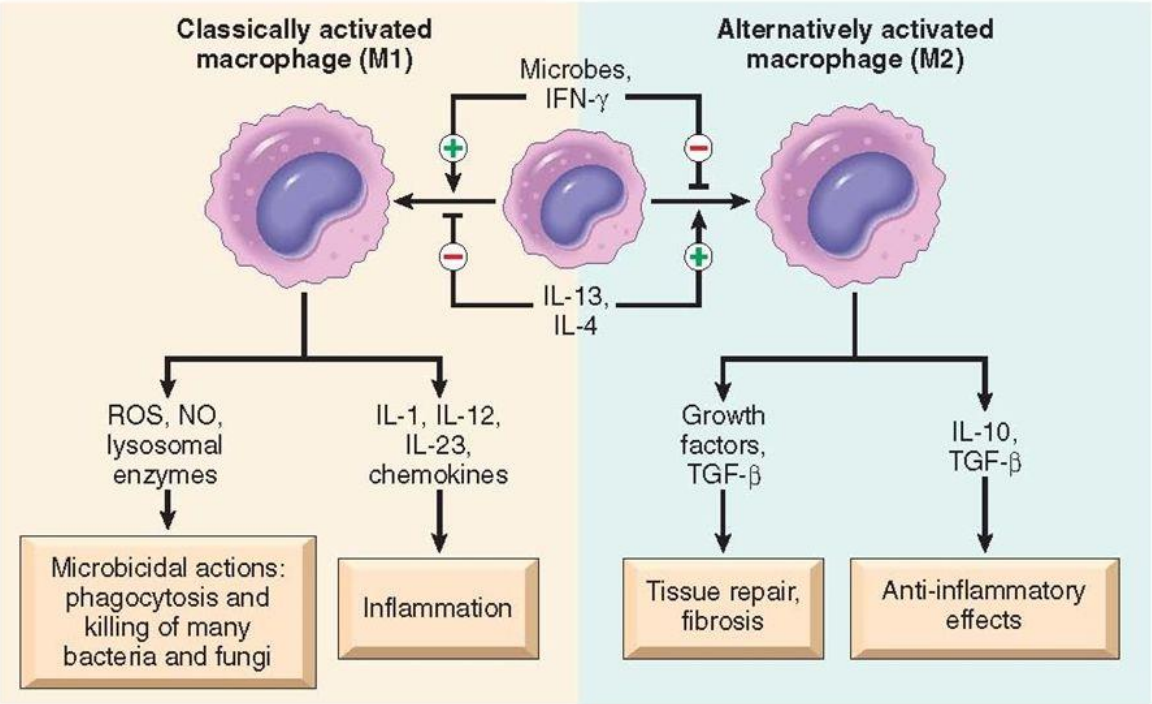
✓ This pathway→ Release of pro-inflammatory cytokines as IL-1, TNF-α, IL-12, IL-23 and stimulation of microbicidal activity.

2. Alternative activation pathway

✓ Its activation occurs in the absence of strong TLR signals and induced by the cytokines IL-4 &IL-13.

✓ This pathway→ Release of IL-10, TGF-β, prolines & polyamines→ Anti-inflammatory effects & wound repair and fibrosis.

Classical and alternative macrophage activation



Mast cells

- Bone marrow derived cells present in skin & mucosal epithelia. Normally not found in circulation. The cytoplasm contains granules.
- The granules contains:
    1. Vasoactive amines as histamine
    2. Proteolytic enzymes
- The cells can be activated by microbial products via TLRs.
- Mast cells express Fc receptors for IgE when the antibody binds the specific Ag→ Release of granules content extracellular.
- Mast cells can synthesize lipid mediators as prostaglandins and cytokines as TNF-α.
- Mast cell activation is responsible for symptoms of allergy and defense against helminthes.

Basophils

- Constitute less than 1% of blood leukocytes.
- Derived from bone marrow cell lineage different from that of mast cells.
- Normally not present in tissues.
- Their cytoplasm contains granules similar to mast cells & can synthesize the same mediators as mast cells.
- Basophils express Fc receptors for IgE.

Dendritic cells

- Considered the most important APC for activation of naïve T cell. They respond to microbes by producing cytokines that initiate inflammation & stimulate adaptive immune response through presenting Ag to naïve T cell. Accordingly, they're considered a bridge between innate and adaptive immune systems.
- Found throughout the body, more concentrated at the portals of microbe entry "skin, lung, GIT"
- Arise from bone marrow from either myeloid or lymphoid lineage.
- They have branch like cytoplasmic projections, express various types of TLR & cytoplasmic PRRs.

- There are 2 types:
  1. *Classical or conventional DCs*: Respond to microbe by migrating to LN to present the Ag after processing with MHC class II to T cell
  2. *Plasmacytoid DCs*: Respond to viral infections and considered important source of type 1 INF.

Activated DC:

- Loses its adhesiveness to epithelia preparatory to migration to regional LN.
- Express CCR_7 receptor specific for the 2 chemokines produced in the T cell zone.
- Express high levels of MHC molecules with bound peptide and co-stimulators "B7.1 & B7.2". So as to be able to stimulate naïve T cell.

Cross presentation

- Some specialized DCs have the ability to ingest virus infected cells & deliver viral proteins into cytosol to be processed & presented to naïve $CD8^+$ with class I MHC molecule. The same cross-presenting APC may display class II MHC–associated antigens from the microbe for recognition by $CD4^+$helper T cells.

Natural killer cells (NK)

- Considered the first & best described innate lymphoid cell (ILC) "a subset of type I ILCs"
- Their major function is killing infected cells similar to cytotoxic T cells (CTLs).
- Constitute 15-25% of mononuclear cells in blood & spleen, appears as large lymphocytes & numerous cytoplasmic granules.
- Identified by the presence of CD56 & absence of CD3. They also express CD16 "FcγRIIIA" involved in recognition of antibody coated cells.

- Main functions:

1. Killing infected cells
2. Secretion of INF-ɣ

NK receptors

1. Killer activating receptors "KAR"
2. Killer inhibitor receptors "KIR"

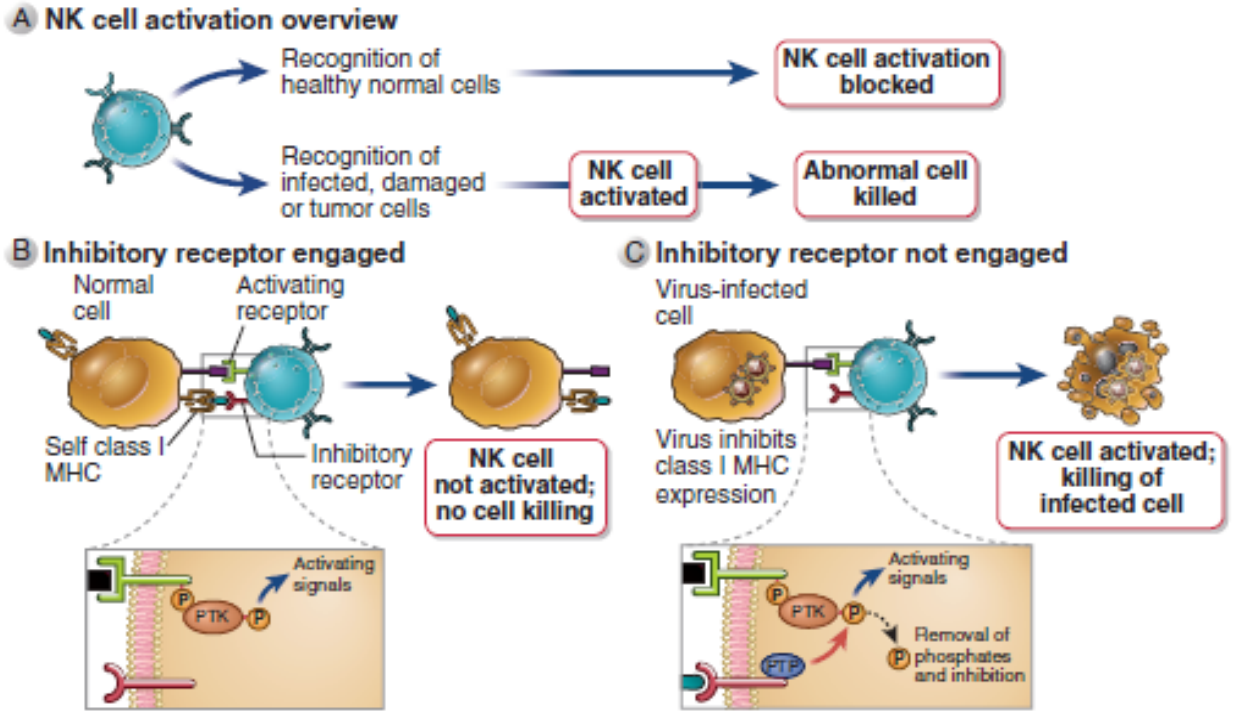
KAR

a. Killer cell Ig like
b. C- type lectin: NKG2D binds MIC-A & MIC-B
c. CD 16 "FcγRIIIA": Specific for Fc portion of IgG→ killing of infected cells coated by these antibodies "ADCC".

KIR

a. Killer cell Ig like receptor recognize MHC class I
b. Lectins such as CD94/NKG2A heterodimer also bind MHC class I
c. Leucocyte Ig like receptors (LIRs)

- ✓ When the inhibitory receptors are engaged with self MHC class I molecules the killing activity of NK cell is shut off.
- ✓ Blocking expression of MHC Class I molecules on host cells, the inhibitory receptors of NK cell will not be engaged→ NK cell activation. This process is called ***recognition of missing self.***



- The mechanism of killing is mediated through ***perforins & granzymes*** like CTLs. Perforin proteins induce pores formation in target cells thus facilitating the entry of granzymes which stimulate the caspase activities→ Death by apoptosis.
- Some NK cells don't express CD16 nor are they cytotoxic but produce abundant IFN-ɣ. They are called *non-toxic NK cells.*
- KIR genes are polymorphic i.e. there are several allelic variants in humans. Some haplotypes are associated with increased susceptibility to some diseases including spontaneous abortions & uveitis.
- A positive feedback loop exists between NK cells and macrophages
  - ✓ NK cells secrete IFN-ɣ which activates macrophages to secrete:
    1. IL-15: Important for development and maturation of NK cells to secrete more IFN-ɣ.
    2. IL-18: stimulates NK cells to secrete more IFN-ɣ.
    3. IL-12 & IFN-1: Enhance the killing function of NK cells.

Innate lymphoid cells (ILCs)

- They arise from a common bone marrow precursor identifiable by the presence of the transcription factor ***Id2.***

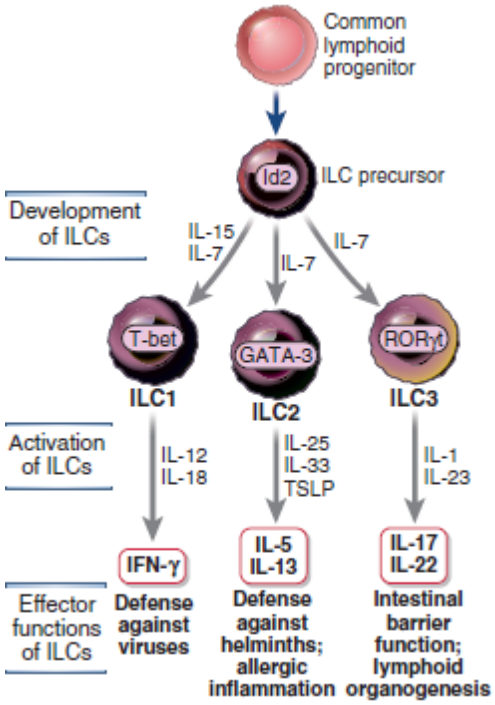
- Unlike lymphocytes of the adaptive immune system:
  - ✓ They emerge fully capable of performing effector functions without the need for clonal expansion and differentiation.
  - ✓ They don't express TCRs.
  - ✓ Their residence at epithelial barriers makes them early responders to microbes that colonize tissues, giving time to effector T cells to differentiate giving rise to a more specific immune response and larger amounts of cytokines.

- They are 3 major groups or subsets identified by the cytokines they produce and the transcription factors

I. **Group 1 "*analogous to Th1*"** express the transcription factor *T-bet* and produce *IFN-ɣ* they include NK cells. ILC1s are likely important for defense against intracellular microbes.

II. **Group 2 "*analogous to Th2*"** express the transcription factor *GATA-3* and produce *IL9, IL5 & IL13*. They contribute to allergic diseases and defense against parasitic infection.

III. **Group 3 "*analogous to Th17*"** express the transcription factor *RoRɣT* and produce *IL22 and/or IL17*. They are found mainly at mucosal sites and share in defense against extracellular bacteria as well as maintaining integrity of epithelial barriers.
  - ✓ **Lymphocyte inducer cells** a "member of group 3 ILCs" expressing molecules that are important in organization of lymphatic tissue. They secrete *lymphotoxin-α & β* which stimulate:
    1. Follicular dendritic cells "FDCs" to secrete the chemokine CXCL13….
    2. Fibroblast reticular cells "FRCs" to secrete CCL19 & CCL21…..



III. Circulating proteins

- In addition to pentraxins, collectins & fecolins previously mentioned complement proteins or ***"complement system"*** are important in innate & adaptive immune responses.
- The complement system consists of 20+ plasma proteins as well as membrane regulators and receptors.
- Proteins of the complement pathway circulate in plasma and are present in lower concentrations in other body fluids as well as intracellular space. The components in plasma are synthesized in the liver, whereas the components in other sites represent a combination of local synthesis and filtration from plasma.
- Complement activation involves rapid self-amplifying proteolytic cascades in which an inactive precursor is altered to become an active protease that cleaves and thereby activates the next complement protein in the cascade causing amplification of the response.
- The first step in activation is recognition of molecules on microbial surface. This activation occurs in 3 ways each called a pathway of complement activation
- Two pathways *"alternative & lectin pathways"* are initiated by microbes in the absence of antibodies; so are components of innate immune system responses. The third called the *"classical pathway"* is initiated by antibodies attached to antigens; so considered a component of adaptive immune system responses".

- Complements of classical pathway are designated by the capital letter C combined with a number. Numbers were in order of discovery not activation (C4 is activated before C3 in classical & lectin pathways).
- Upon proteolytic cleavage, a liberated smaller fragment is designated by 'a' small letter such as C3a. The larger fragment is attached and is then listed with a 'b' such as C3b.
- Alternative pathway components are designated as factors such as factor B & factor D.
- Inactivation occurs by limited proeteolysis. Further products are designated by suffixes (e.g C3b is cleaved giving rise to C3c & C3d).
- The membrane receptors & regulators are present on hematopoietic cells and therefore have been given 'cluster of differentiation' assignments (e.g. CR1, CD35).

- ***The alternative pathway*** is triggered when a breakdown product of C3 hydrolysis, called C3b is deposited on the surface of a microbe. Thus bound C3b binds another protein called factor B which is broken down by plasma protease generating Bb fragment forming *C3bBb* ***"C3 convertase"***. Properdin stabilizes C3 convertase preferentially on microbial surfaces over normal host cells. C3 convertase creates many more C3 → *C3bBb3b* ***"C5 convertase"***→ Breakdown of moreC5.

- ***The classical pathway*** is triggered when IgM or IgG1, IgG2 or IgG3 bind to antigens→ The Fc portion of the Ab becomes accessible to & binds the C1 complement protein {C1q "binding component; C1r & C1s "proteases"}
  - ✓ These proteases become enzymatically active and lead to cleavage of C2 & C4 forming *C4b2a* ***"C3 convertase"*** which function to breakdown more C3 & the generated C3b binds to the C4b2a forming *C4b2a3b* ***"C5 convertase".***

- ***The lectin pathway*** is activated when MBL binds to terminal mannose residues on the surface glycoproteins of microbes. This binding activates serine proteases called "MASP 1& 2" with subsequent cleavage of C4 & C2. Rest of steps as classical pathway.

- The later steps of activation of ***MAC "membrane attack complex":*** C5 convertase activates cleavage of C5 & C5b fragment recruits sequential complexes formed by C6, C7, C8 and polymers of C9. The C9 polymers make multiple holes or pores in the cell membrane through which water and ions enter→ Death of the microbe.

N.B$_1$: Activated complement proteins become covalently attached to the cell surface where the activation occurs so the complement effector functions are limited to the correct site.
N.B$_2$: The complement system is tightly regulated by molecules present on host cells and plasma so prevents uncontrolled and potentially harmful complement activation.

Regulatory proteins

- Nearly one half of complement proteins serve a regulatory function. The goal of regulation is to prevent complement damage to normal host tissue.
- Regulatory proteins act by destabilizing activation complexes and by mediating specific proteolytic cleavage to degrade fragments derived from activation. The complement is regulated at the following critical steps:
  - ✓ Activation or inhibition
  - ✓ Amplification (convertase formation)
  - ✓ Membrane attack

- *Examples of regulatory proteins:*
  1. In plasma:
     - C1 inhibitor (C1INH)
     - Factor I
     - Factor H
     - C4 binding protein (C4BP)

  2. On cell membrane
     - Membrane cofactor protein (MCP, CD46)
     - Decay accelerating factor (DAF)
     - CD59
     - Type 1 complement receptor (CR1, CD35)

Example: C1 inhibitor binds to and inactivates the protease components of C1. "C1 inhibitor/C1r/C1s" complex dissociates from C1q remains bound to the immune complex.

- The effector functions of complement activation:
  1. Opsonization & phagocytosis: C3b acts as opsonin
  2. Cell lysis through MAC
  3. Stimulation of inflammatory reaction: through the small peptide fragments C3a, C4a & C5a which are chemotactic for neutrophils and stimulates the release of inflammatory mediators from various leukocytes.

IV. Cytokines

- Cytokines are soluble proteins of low molecular weight that mediate immune & inflammatory functions.

- Nomenclature
  - ✓ Many cytokines are named using the name 'interleukin' (between WBCs).
  - ✓ Some cytokines are still denoted by their descriptive names (e.g. Colony stimulating factor {CSF}, Tumor necrosis factor {TNF}, Interferon {IFN}, Platelet derived growth factor {PDGF}, Fibroblast growth factor {FGF}) even though these descriptive names usually represent one of many activities.

- They have the following properties:
  - ✓ They signal between cells and coordinate the immune response (i.e. they are the hormones of the immune system). Hence, they play an integral role in the initiation, perpetuation and subsequent down regulation of the immune response.
  - ✓ They have autocrine (effect on the cell producing the cytokine), paracrine (effect on cells in the vicinity) or if produced in large quantities endocrine activities (effect on distant sites).
  - ✓ They are also produced by non-immune cells such as fibroblasts and endothelial cells.
  - ✓ Cytokines are synthesized as required i.e. each time their synthesis is initiated by new gene transcription and never pre-formed or stored.
  - ✓ Secretion is brief and self-limited.
  - ✓ Their action is not antigen specific.
  - ✓ They bind to high affinity receptors.

  - ✓ Cytokines are highly interactive. Their actions may be:
    1. **Pleotropic**: Act upon different cell types bringing different biological effects in each cell type.
    2. **Redundant**: Different cytokines mediate similar functions.
       - The previous 2 features are more evident with cytokines secreted with the adaptive immune response "IL-2, IL-4….."
    3. **Synergistic**: Combined effect of 2 or more cytokines on cellular activity greater than the combined effect of the cytokines acting separately.
    4. **Antagonistic**: Effect of one is opposite to the other.
    5. **Cascade effect:** One cytokine enhances or inhibits the production of another cytokine.

Cytokines of the innate immune system

- Mainly produced by macrophages and dendritic cells.
- Main role in innate immune responses:
    1. Induce inflammation: IL-1, IL-6, TNF
    2. Inhibiting viral replication:IFN-1
    3. Promoting T cell responses:
        - ✓ IFN-ɣ→++Th1
        - ✓ IL-23→Maintain Th-17
        - ✓ IL-27→++Th1 & --Th17
        - ✓ IL-15→++CD8 memory cells
    4. Promoting NK cell function & differentiation: IL-12, IL-15, IL-18
    5. Anti-inflammatory effect: IL-10, TGF-β
    6. Promote wound healing and tissue regeneration: Tumor growth factor-β (TGF-β), FGF, Vascular endothelial growth factor (VEGF), PDGF.
    7. Stimulate the production of further leukocytes: GM-CSF, G-CSF, M-CSF.

Specific features for some cytokines:

TNF

- On hypothalamus→ fever
- Induce prostaglandins, metalloproteinases.
- Increase the expression of adhesion molecules.
- If produced in large quantities:

1. Inhibit cardiac contractility
2. Inhibit vascular smooth muscle tone→ Vasodilatation
3. Enhance thrombus formation
   If these changes are severe →shock & DIC

N.B: SIRS= Systemic inflammatory immune response

- Similar to septic shock. Seen with burns, trauma, pancreatitis & other serious conditions.
- Signaling through TNF is mediated via binding to 2 receptors (TNF I, TNF II).
- Binding to these receptors leads to recruitment of proteins called TNF receptor associated factors (TRAFs) which eventually activate the transcription factors NFκβ & AP1.
- Signaling through TNFR I may also lead to activation of caspases→ Apoptosis.

IL-1

- The biologically active form is IL-1β.
- On hypothalamus→ fever by increasing synthesis of prostaglandins (PGs).
- On liver→ increase the synthesis of acute phase reactants.
- Increases the expression & affinity of adhesion molecules.
- Leucocyte activation.
- Stimulates leukocyte production from bone marrow.

IL-12

- Secreted from DC & potentiates differentiation of T cells to Th1
- IL-12 is formed of 2 subunits (p35 "35 KD peptide", p40)
- P40 is also present as a component of IL-23 which activates Th17
- *So antibodies blocking p40 will block both →IL-12 dependent Th1 & IL-23 dependent Th17.*

TGF-β

- Fibroblast proliferation, collagen and tissue inhibitor of metalloproteinase (TIMP) synthesis.
- Decrease metalloproteinases.
- Angiogenesis.
- Decreases pro-inflammatory cytokines.
- Differentiation of T-regulatory cells.

TABLE 4.5 Cytokines of Innate Immunity

Cytokine	Size	Principal Cell Source	Principal Cellular Targets and Biologic Effects
TNF	17 kD; 51 kD homotrimer	Macrophages, T cells	Endothelial cells: activation (inflammation, coagulation) Neutrophils: activation Hypothalamus: fever Muscle, fat: catabolism (cachexia) Many cell types: apoptosis
IL-1	17 kD mature form; 33 kD precursors	Macrophages, endothelial cells, some epithelial cells	Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever Liver: synthesis of acute-phase proteins T cells: T_H17 differentiation
Chemokines (see Table 3.2)	8–12 kD	Macrophages, endothelial cells, T cells, fibroblasts, platelets	Leukocytes: chemotaxis, activation; migration into tissues
IL-12	Heterodimer of 35 kD and 40 kD subunits	Macrophages, DCs	T cells: T_H1 differentiation NK cells and T cells: IFN-γ synthesis, increased cytotoxic activity
Type I interferons (IFN-α, IFN-β)	IFN-α: 15–21 kD IFN-β: 20–25 kD	IFN-α: macrophages, plasmacytoid DCs IFN-β: fibroblasts	All cells: antiviral state, increased class I MHC expression NK cells: activation
IL-10	Homodimer of 34–40 kD and 18 kD subunits	Macrophages, T cells (mainly regulatory T cells)	Macrophages, DCs: inhibition of expression of IL-12, costimulators and class II MHC molecules
IL-6	19–26 kD	Macrophages, endothelial cells, T cells	Liver: synthesis of acute-phase proteins B cells: proliferation of antibody-producing cells T cells: T_H17 differentiation
IL-15	13 kD	Macrophages, others	NK cells: proliferation T cells: proliferation (memory $CD8^+$ cells)
IL-18	17 kD	Macrophages	NK cells and T cells: IFN-γ synthesis
IL-23	Heterodimer of unique 19 kD subunit and 40 kD subunit of IL-12	Macrophages and DCs	T cells: maintenance of IL-17–producing T cells
IL-27	Heterodimer of 28 kD and 13 kD subunits	Macrophages and DCs	T cells: T_H1 differentiation; inhibition of T_H17 cells NK cells: IFN-γ synthesis

DC, Dendritic cells; *MHC*, major histocompatibility complex; *IFN*, interferon; *IL*, interleukin; *TNF*, tumor necrosis factor. (Also see Appendix I.)

- Signaling pathways for most cytokines start after binding to cytokine receptors type I or II with recruitment and activation of ***Janus Kinases (JAKs), JAK 1, JAK 2, JAK 3*** and subsequent activation of ***STATs "signal transducers and activators of transcription***". These transcription factors are responsible for the production of various molecules (other cytokines, chemokines, co-stimulatory proteins ….) that mediate & control the immune responses.
- JAKs are now targets for therapeutic applications.
- Cytokine signaling may be inhibited by:
  - ✓ SHP (SH2 cotaining phosphatase)
  - ✓ PIAS (Protein inhibition of activated STATs)
  - ✓ SOCS (Suppressor of cytokine signaling)

Chemokines

- Include a large family of 8-10 KD cytokines. The name "chemokine" is a contraction of "chemotactic cytokine"
- Functions:

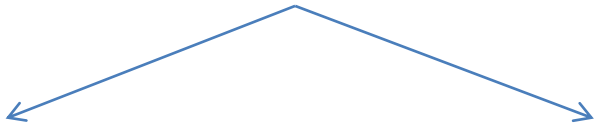
1. Stimulate leukocyte movement to regulate the migration of leukocytes from blood to tissues.
2. Aid in development and maintenance of lymphoid tissue architecture.
3. Control the migration of DCs from site of infection to draining lymph nodes.

Classes or families

- Classified on the basis of the number and location of N-terminal cysteine residues.

1- CC: 2 defining cysteine residues are adjacent.
2- CXC: called also α family in which the 2 cysteine residues are separated by one amino acid.
   - ✓ These 2 families are the major.
   - ✓ CXC family is chemotactic for neutrophils whereas CC family is chemotactic for monocytes and both are chemotactic for T cells.
3- C: C family contains one cysteine residues.
4- CX_3C: 2 cysteine residues separated by 3 amino acids.

The two main reactions of the innate immunity are:



Inflammatory response ***Antiviral response***

Inflammatory response

- Release of pro-inflammatory cytokines.
- Acute phase reactant response.
- Recruitment of leukocytes to site of infection.
- Ingestion & killing of microbes by activated phagocytes "phagocytosis".

Acute phase reactant response

- Acute phase proteins are a class of proteins whose plasma concentrations increase "+ve acute phase proteins" or decrease "-ve acute phase proteins" by at least 25% in response to inflammation whether acute or chronic. They can occur in association with a wide variety of disorders including infection, trauma, infarction, inflammatory arthritidis and various neoplasms.

- Mechanism:

✓ Inflammatory cells →Release of IL-1 & TNFα→ Liver to increase the synthesis of some proteins "+ve" on the expense of other proteins "-ve"

- Acute phase proteins:
  1. CRP, Serum Amyloid P (SAP), MBL: Act as opsonins.
  2. Complement proteins: opsonization, lysis,chemoattractant
  3. Fibrinogen, prothrombin, Favtor VIII: Trap invading microbes in blood clots. Some act as chemoattractants.
  4. Plasminogen: Degradation of blood clots.
  5. α2-macroglobulin: Inhibitor of coagulation by inhibiting thrombin and inhibitor of fibrinolysis by inhibiting plasmin.
  6. Ferritin: Binding iron to inhibit microbial iron uptake.

7. Hepcidin: Stimulates the internalization of ferroprotein.
8. Ceruloplasmin: Oxidizes iron facilitating the action of ferritin.
9. Haptoglobin: Binds hemoglobin.
10. Orosomucoid: Steroid carrier.
11. Alpha-1 antitrypsin & Alpha-1 antichemotrypsin: Down-regulates inflammation.

- Negative acute phase reactants
  1. Albumin
  2. Transferrin
  3. Transthyretin
  4. Transcortin
  5. Retinal binding protein
  6. Antithrombin

✓ The decrease of their levels may be used as a marker of inflammation.

CRP

- CRP can influence multiple stages of inflammation. It has both pro-inflammatory and anti-inflammatory actions.
- A major function of CRP is its ability to bind phosphocholine, thereby permitting recognition of both foreign pathogens that display this moiety and of phospholipid constituents of damaged cells.
- Pro-inflammatory effects:
  - ✓ CRP can activate the complement system and can bind to phagocytic cells via Fc receptors resulting in release of inflammatory cytokines and tissue factor.

- Anti-inflammatory effect:
  - ✓ Fewer neutrophils accumulate at inflammatory sites because of reduced ability of the neutrophil to adhere to endothelium. Another function is enhancement of apoptotic cell clearance by CRP.

ESR

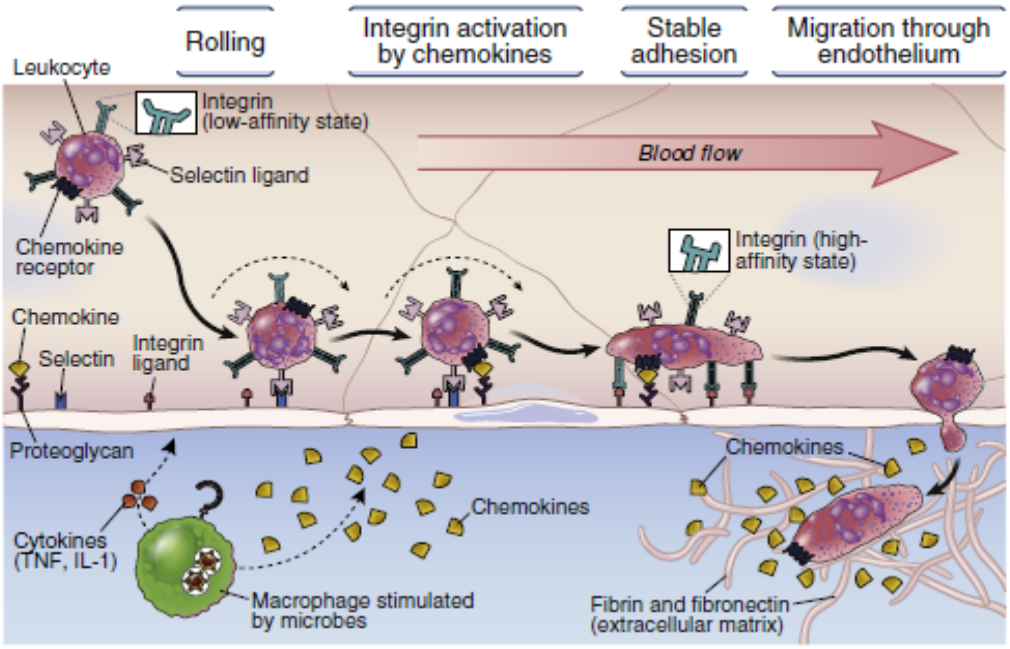
- ESR is not a protein but reflects plasma viscosity and the presence of acute phase proteins, as well as other influences.

Recruitment of leukocytes to sites of infection

- From blood to site of infection or tissue damage.
- **Mechanism**: Binding to venular endothelial adhesion molecules & extravasation following the high concentration gradient of chemoattractants produced at site of infection.

- Steps:

1. ***Selectin mediated rolling***
   - ✓ In response to TNF α& IL-1 secreted at site of infection→ Expression of E-selectin & P-selectin on endothelial cells. Those will weakly bind to selectin ligands on leukocytes, the blood flow will disrupt this binding & the bonds reform downstream and so on.
2. ***Integrin mediated firm adhesion***
   - ✓ Chemokines produced at site of infection from both tissue macrophages and endothelial cells→ increase the affinity of integrin molecules on leukocytes.
   - ✓ TNF α& IL-1 stimulate endothelial cells to express integrin ligands→ Firm binding of integrins to theirligands→ Arrest the rolling leukocytes which spread out on endothelial surface.
3. ***Transendothelial migration of leukocytes along concentration gradient***
   - ✓ Increases the concentration of chemokine at site of infection will attract leukocytes which have these chemokine receptors.



Phagocytosis & killing of microbes

- The main phagocytic cells: *Macrophages & Neutrophils*
- They defend the body by ingesting & destructing microbes in addition they remove cellular debris arising from normal physiologic functions "cleansing mechanism".
- Other phagocytic cells are DCs & B lymphocytes, they engulf microbes but for a different aim "see later"

- Steps:

1. **Recognition of the microbes & binding to membrane receptors** e.g.:
   - PRRs
   - Fc receptors
   - Complement receptors
2. I**ngestion**:
   - ✓ Extension of the phagocyte plasma membrane around the microbe forming a vesicle called phagosome that fuses with lysosome forming phagolysosome.
3. **Destruction by activation** of the following enzymes:
   - Phagocyte oxidase: converts molecular O2 to superoxide anions & free radicals "*oxidative burst".*
   - Inducible nitric oxide synthase "iNOS": catalyzes the conversion of arginine to nitric oxide (NO).
   - Myeloperoxidase→ Formation of hypochlorus acid (HOCL).
   - Lysosomal proteases that break down microbial proteins.
   - ✓ All these microbicidal substances are produced mainly within the lysosomes & phagolysosomes acting on the microbes with no damage to the cell itself.

Chronic granulomatous disease:

- ✓ Inherited deficiency of phagocyte oxidase enzyme in which phagocytes are unable to eradicate infection. Recruitment of more macrophages and leukocytes as a trial of the immune system to eradicate infection will lead to collection of these cells around the microbe forming ***"granuloma".***

Antiviral response

- Mediated mainly by type-1 interferons.
- Type 1 interferons are a large family of structurally related cytokines that mediate the early innate immune repsonses to viral infections.
  - ✓ IFN-α "13 different closely related proteins produced mainly from plasmacytoid DCs"
  - ✓ IFN- β "A single protein produced mainly from fibroblasts".

- Type 1 interferons bind to interferon receptors (IFN R1& R2) and activated ***$STAT_1$, $STAT_2$ & IRF_9***→ Expression of various genes whose protein products→ *Antiviral response* in many ways:

1. Double stranded RNA-activated serine/threonine protein kinase (PKR) → Blocks viral transcriptional & translational events.
2. 2',5'oligoadenylesynthetase&RNase→ Viral RNA degradation.
3. Induction of CD69 on lymphocytes with decreased expression of SIP receptors→ sequestration of lymphocytes in LNs.
4. Increase cytotoxicity of NK cells & CD8 and promote Th1.
5. Up-regulation of expression of MHC class I molecules. Protection against viruses is due in part to the activation of intrinsic apoptotic death pathways & enhanced sensitivity to extrinsic inducers of apoptosis like TNF-induced apoptosis.

Stimulation of adaptive immunity by innate immune system

1. Dendritic cells: The professional cell needed to activate naïve lymphocytes.
2. Molecules needed for the 2^{nd} signals:
   - ✓ B7.1 & B7.2→ Binds CD28 expressed on DCs & macrophages.
   - ✓ C3d fragment→ Binds complement receptor on B lymphocyte "CR2, CD21".
3. Cytokines produced during innate immune response promote and modify differentiation of effector lymphocytes during adaptive immune responses, e.g.:
   - IL-12→ Th1 differentiation.
   - IL-1, IL-6 & IL-23→ Th17 differentiation.
   - IL-15→ Survival of memory CD8 cells.
   - IL-6→ Production of antibodies by activated B cells.

- **Homeostatic mechanisms of innate immune response include the following:**

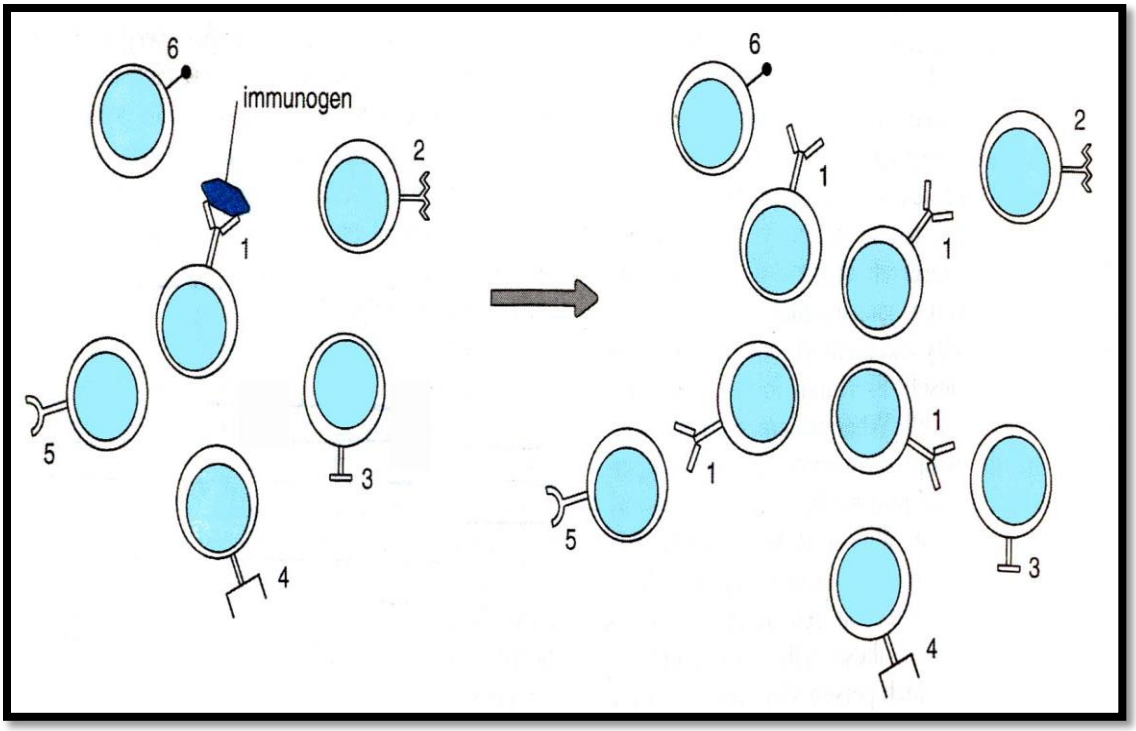
1. Upon decrease of antigen load the macrophage activation is shifted to the alternative pathway with the production of the anti-inflammatory cytokines IL-10 & TGF-β that inhibit the production of various pro-inflammatory cytokines "IL-1, TNF, IL-12" from activated macrophages and DC cells so considered a *negative feedback regulator*. IL-10 is also produced by T-reg.
2. Phagocytes also produce **IL-1 receptor antagonist (IRA)** which is structurally similar to IL-1 but biologically inactive, binding to the receptor and acting as a competitive inhibitor.
3. **SOCS** "Supressor of cytokine signaling proteins" supressors of JAK-STATs signaling pathways SOCS expression is induced by signaling via TLR.
4. **Autophagy** proteins also impair cytokine signaling. Targeted mutations in different autophagy genes result in increased synthesis of IL-1 & IL-18 and the development of inflammatory bowel syndrome.
5. The complement component C1q, collectins and mannose binding lectin can bind to apoptotic cells and mediate their clearance.

- ✓ Macrophages also control inflammation through their ability to rapidly ingest and clear apoptotic cells, the process of **"efferocytosis".**
- ✓ Apoptotic cells and autolyzed necrotic neutrophils release cytotoxic pro-inflammatory constituents including toxic enzymes, oxidants, proteases and caspaces in the environment. Upon recognition of apoptotic cells through various DAMP like phosphatidyl serine or calreticulin, macrophages release anti-inflammatory cytokines, anti-protease and growth promoting factors. These growth factors are thought to promote the replacement of dead cells.
- ✓ Efferocytosis can be performed by many other cell types including epithelial cells and fibroblast.
- ✓ Impaired efferocytosis has been linked to autoimmune diseases such as SLE, cystic fibrosis and asthma.

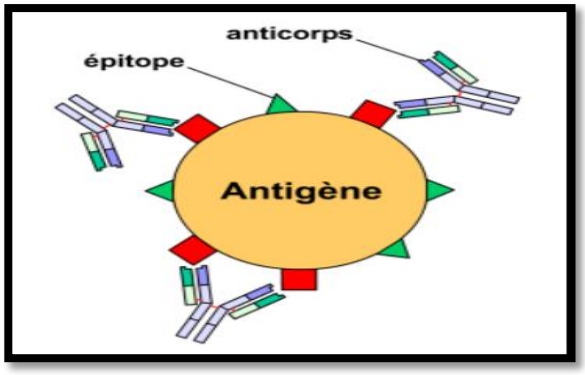
Adaptive immune system

Components

- T & B lymphocytes and their products: antibodies, cytokines & effector molecules.
- Adaptive immunity is the form of immunity that is mediated by lymphocytes in contrast to innate immunity. It is responsible for later response mechanisms against infectious agents. It develops more slowly but more effective. It is also characterized by *memory* which is the ability to respond vigorously to repeated exposures to the same microbe.
- The adaptive immune system is triggered if the antigen passes through the epithelia and delivered to the secondary lymphoid organs.
- Recognition is carried out through the antigen binding receptors of T & B lymphocytes which are the T cell receptor and the B cell receptor.
- These receptors show more diversity than receptors of the innate cells (>a billion antigenic specificity).
- The receptors can recognize and react to numerous microbial antigens, microbial substances as well as non-infectious molecules.
- T & B lymphocytes arise from a subset of HSC in the bone marrow that becomes committed to the lymphoid pathway "***lymphoid lineage".***
- B lymphocytes complete their development in the bone marrow & spleen while T lymphocytes complete their development in the thymus. They emerge in a quiescent state called resting, mitotically inactive or **naïve *lymphocytes***.
- Histologically lymphocytes appear identical to each other. They can be distinguished by the functional properties and by the specific molecules they express.
- The hemopoietic system is capable of producing lymphocytes with diverse antigenic specificities. It is about 10^{9} distinct specificities of BCR &>$10^{10}$for TCRS. This range is known the primary ***lymphocyte repertoire.***
- Accordingly lymphocytes ***are precomitted*** in their immune specificities before interactions with antigens.
- Each Lymphocyte or Clone of lymphocyte has a uniquely restricted specificity for antigens: the BCRS or TCRs expressed on cells of a given clone are identical and can respond only to the limited set of antigens recognized by them, this is known as ***"clonal restriction " theory.***
- Whenever anyone of these cells encounter its specific antigen under conditions that favor activation. it can give rise to multiple daughter cells ***"clonal selection***": exposure to antigen selectively promotes the growth of any clone that recognizes it .



- About antigens: Although all antigens are recognized by specific lymphocytes or antibodies. Only some are capable of inducing activation of lymphocytes those are called ***immunogens.***
- Macromolecules such as proteins, polysaccharides or nucleic acids are two big than the antigen binding site of Ab or BCR which binds only a portion or portions of their macromolecules. The bound part is called determinant or epitope. Macromolecules usually contain ***repeated determinants or epiptops***. These capable of inducing cross linkage of many BCRs→ Good response even without the need for T help.

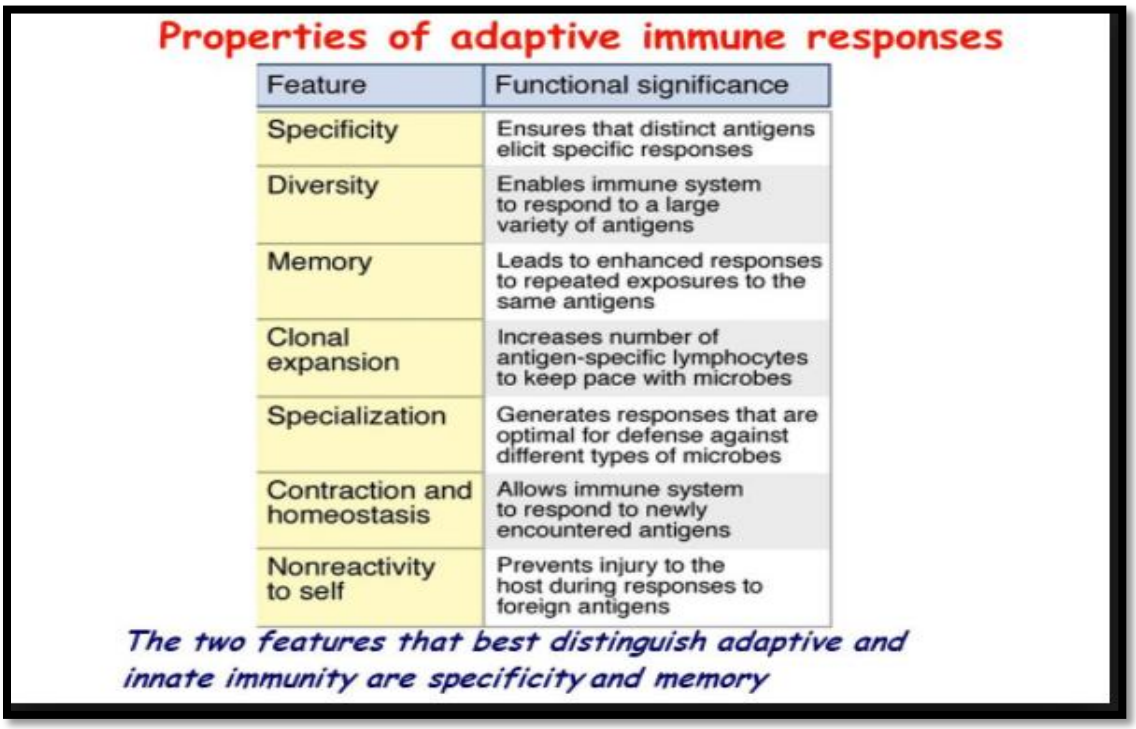


- Epiptopes formed by several ***adjacent amino acid*** residues are called linear determinants. In other cases the determinants may reflect tertiary structure or **conformational *(shape)***

- Proteins may be subjected to modification by: glycosylation, acetylation, phosphorylation, ubiquitination or proteolysis: appearance of new ***epitopes " new antigenic determinants "***
- The strength of binding of antigen to a single Ab or BCR is called ***affinity*** and the overall strength is called ***avidity.***

General features of adaptive immune response:

- ✓ **Specificity:** The system is capable of distinguishing among millions of different of antigens or portions of antigens.
- ✓ **Diversity:** The system can respond to a large variety of antigens.
- ✓ **Memory**: Rapid and enhanced response to repeated exposures to the same antigen.
- ✓ **Clonal expansion**: increase number of only antigen specific lymphocytes.
- ✓ **Specialization:** generated optimal & suitable response to different types of invaders.
- ✓ **Homeostasis:** returning the system to the set point to be able to respond to new antigens.
- ✓ **Non-reactivity to self.**



B lymphocyte

- The principal cell type involved in humoral adaptive immunity: protective effects that are mediated through tissue fluids .
- The main function is to secrete antibodies into blood and other body fluids making them inhospitable to foreign invaders.
- Other functions: APC, Secretion of Cytokines
- When activated they differentiate to **effector *cells, plasma cells*** that secrete Abs and ***memory cells.***

T-lymphocyte

- The principal cell type involved in cell mediated adaptive immunity whose role to combat infections by intracellular microbes.
- Unlike Igs, TCR are never secreted so T cells lack the ability to strike their targets at long distance. Instead they exert their protective effects either through direct contact with a target or by influencing the activity of other immune cell through cytokines.
- Unlike B cells. T lymphocytes recognize a foreign protein only if it is first cleaved into small peptides which are displayed on the surface of APC with either class I or class II MHC molecules : it is the combination of a peptide and MHC molecule that can be recognized by a TCR.
- TCR are always expressed in conjunction with transmembrane surface polypeptide collectively known as CD3 complex (CD3+Zeta chain)***. CD3 complex*** is comparable to Igα and Igβ polypeptide that associate Ig receptor on B lymphocyte. Both function as ***signal transduction molecule.***
- Antigen recognition receptors of B & T lymphocyte.

BCR

Composed of:

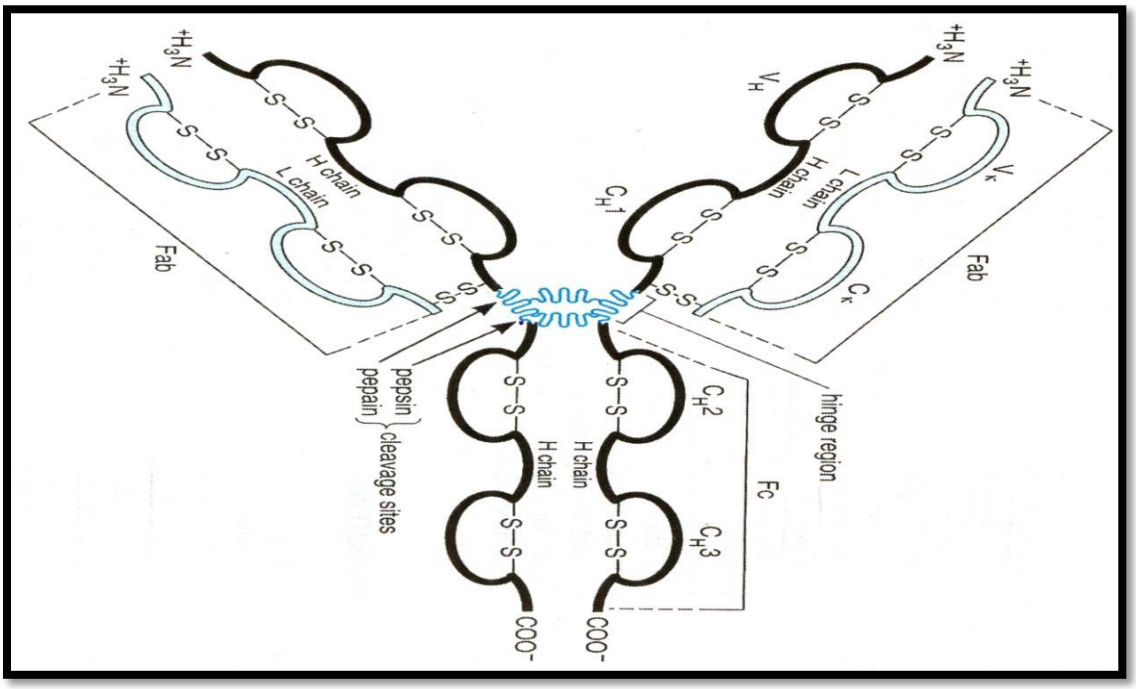
1. Plasma membrane Ab molecule: Ag recognition
2. Invariant 2 polypeptides Ig alpha and Ig B: For signaling transduction.

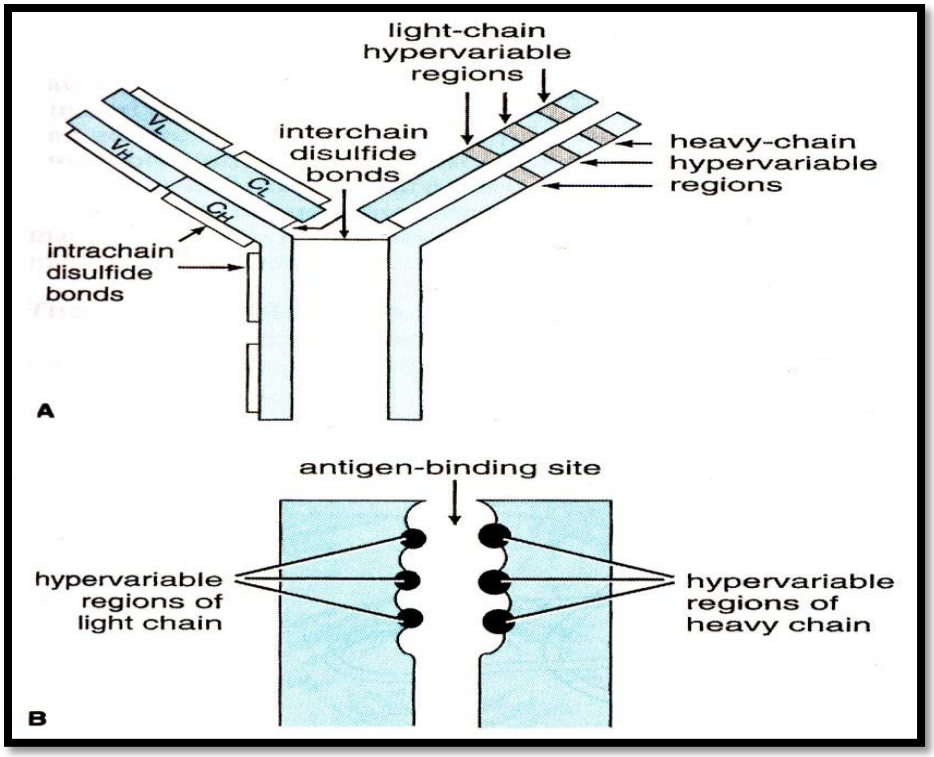
Structure of Ab molecule:

- ✓ 4 polypeptide chains :
  - ***2 heavy (H) : μ, δ, γ, ε ,α***
  - ***2 light (L) : k, λ***
- ✓ Both chains contain a variable region and a constant region.

- ✓ Assembled to form a Y shaped molecule.
- ✓ Each light chain is attached to one heavy chain & the 2 heavy chains are attached to each other all by disulfide bonds .
- ✓ Each light chain is formed of one variable domain and one constant domain while the heavy chain contains one variable domain and 3 or 4 constant domains.
- ✓ Each domain fold in a characteristic 3 dimensional shape called Ig domain shared by other molecules so considered belonging to Ig super family.
- ✓ The antigen binding site is composed of the variable regions of both the heavy and light chains.
- ✓ Each variable region contains 3 hyper variable regions or complementary determining regions (CDR) of which the 3rd one (CDR3) has the greatest variability.

- ✓ The Ig molecule is divided functionally into 2 fragments
    1. Fab (Fragment Ag binding) → Antigen binding.
    2. FC (Fragment crystalline) → Effector functions with a flexible portion called hinge region between both fragments.





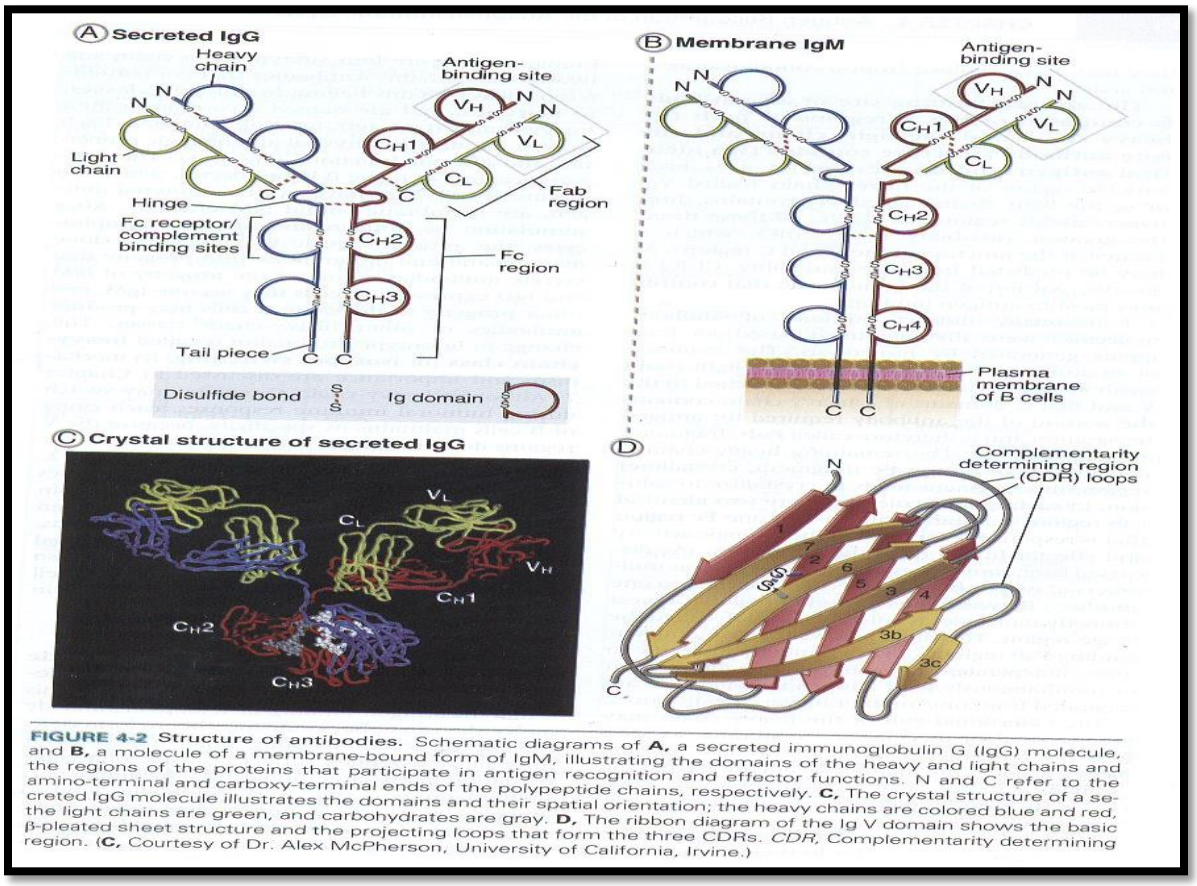
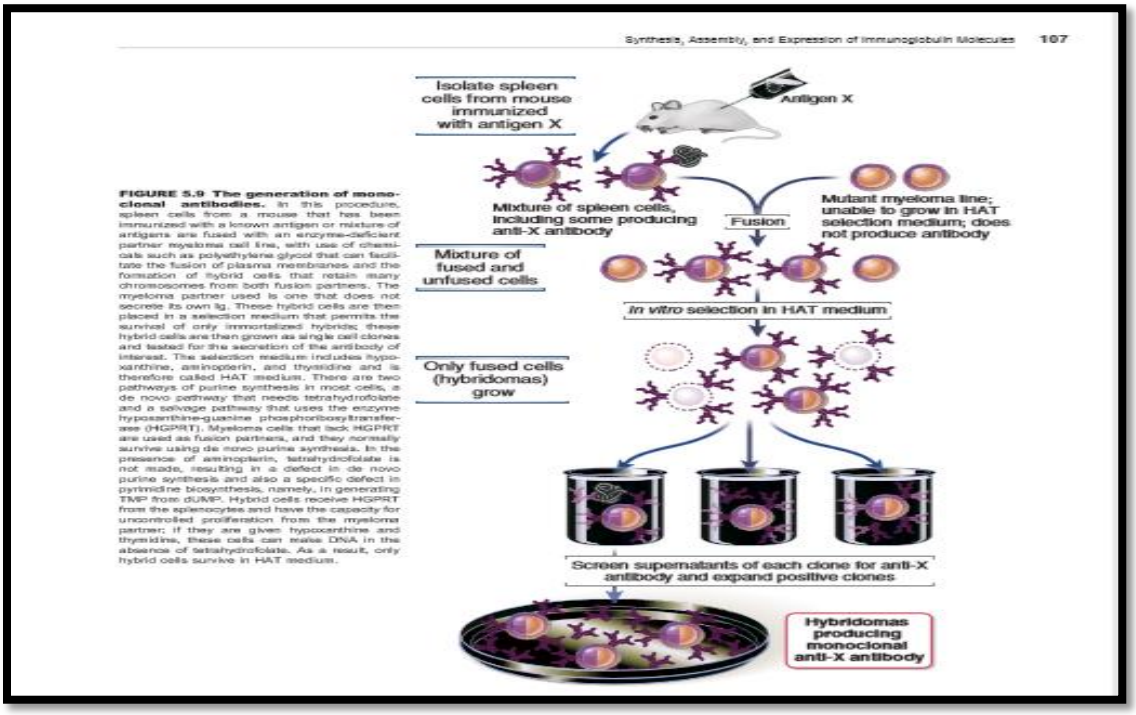


FIGURE 4-2 Structure of antibodies. Schematic diagrams of **A,** a secreted immunoglobulin G (IgG) molecule, and **B,** a molecule of a membrane-bound form of IgM, illustrating the domains of the heavy and light chains and the regions of the proteins that participate in antigen recognition and effector functions. N and C refer to the amino-terminal and carboxy-terminal ends of the polypeptide chains, respectively. **C,** The crystal structure of a secreted IgG molecule illustrates the domains and their spatial orientation; the heavy chains are colored blue and red, the light chains are green, and carbohydrates are gray. **D,** The ribbon diagram of the Ig V domain shows the basic β-pleated sheet structure and the projecting loops that form the three CDRs. *CDR,* Complementarity determining region. (**C,** Courtesy of Dr. Alex McPherson, University of California, Irvine.)

Monoclonal antibodies

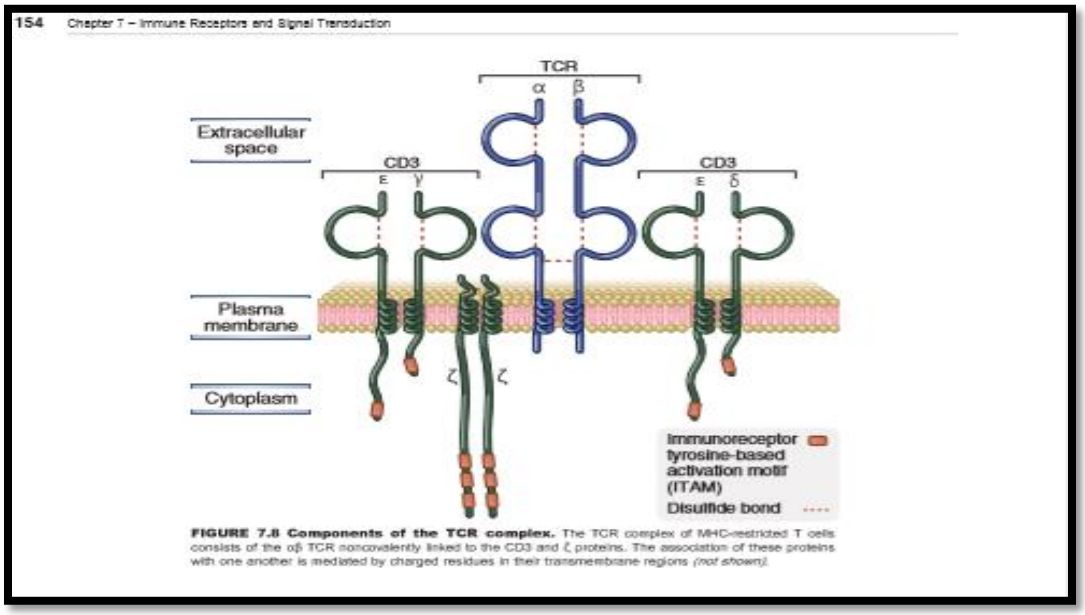
- The realization that ***one clone of B cells*** produce ***only an antibody of only one specificity*** led to the most important technical advance in immunology which is the production of monoclonal antibodies.
- Monoclonal antibodies have vast implications in diagnosis, research and therapy.
- The techniques of production have developed greatly starting from fusion of myeloma cell to B lymphocyte making hyberdomas with subsequent selection of the desired clone to insertion of a complementary DNA, gene manipulation and humanized antibody or totally human monoclonal antibodies .

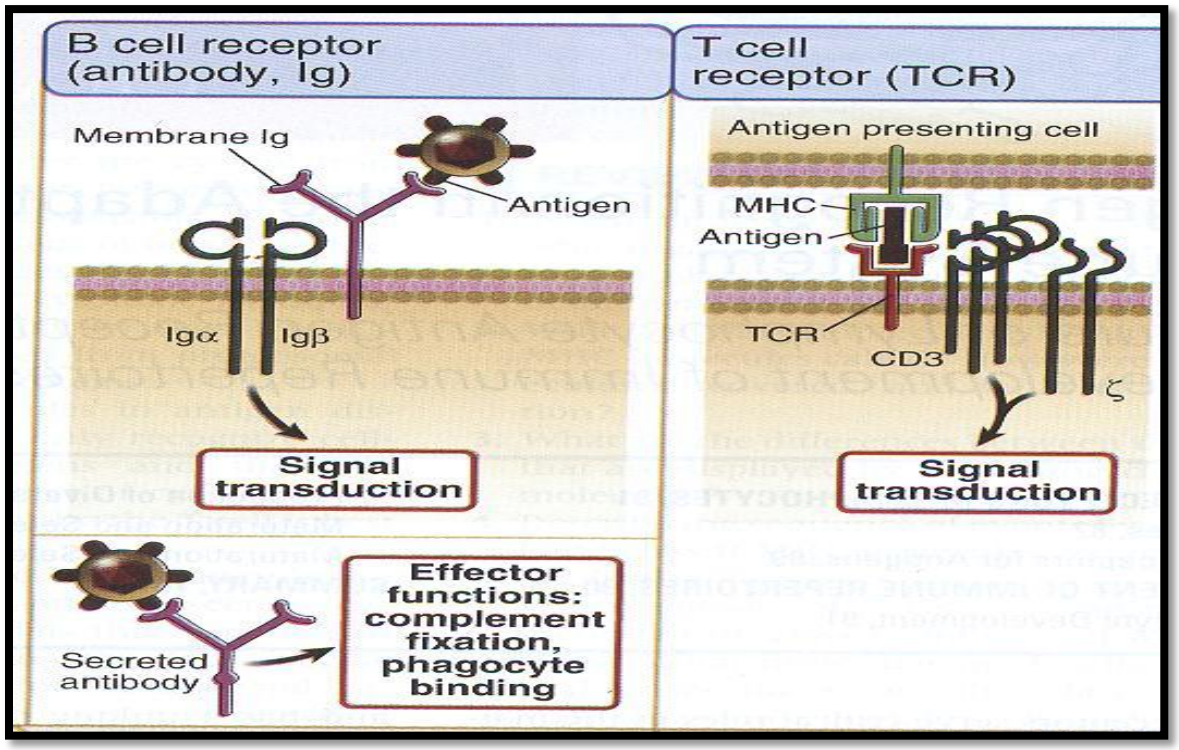


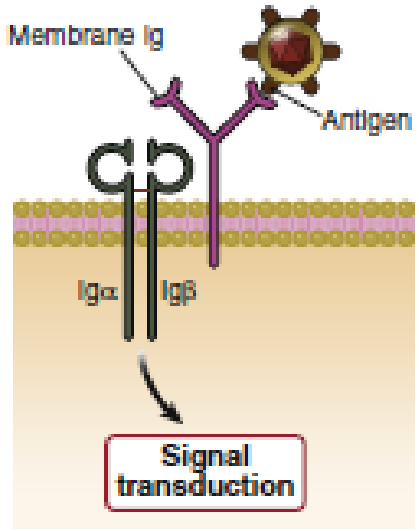
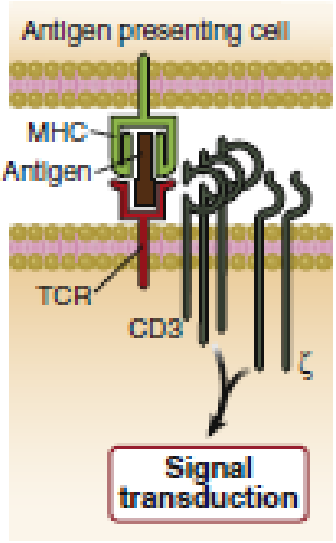
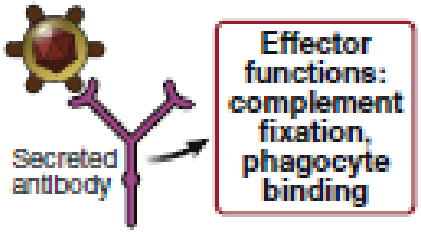
T cell receptors (TCR)

- TCR is a heterodimer formed of an α chain and β chain both chains are anchored in the cell membrane and never produced in a secreted form
- It recognizes ***only peptides bound to MHC molecules*** in case of T helper cell the peptide is presented with MHC class II, in case of T cell cytotoxic the peptide is presented with MHC class I
- After binding, the transmission of signal of stimulation is carried out by CD 3 complex. Full activation also necessitates the interaction of CD4 or CD 8 to the corresponding MHC molecule.

- Only 5-10 % of T –lymphocyte express receptors with gamma and delta chains instead of α and β. These cells are said to be responsible for fast responses specially in mucosal tissues .
- Similar to Ig molecule, each chain is formed of one constant and one variable domain. The antigen binding site is formed by the variable domain of α chain and the variable domain of β chain. Each variable domain contains hyper variable "CDRS" regions with CDR3 showing the greatest variability.





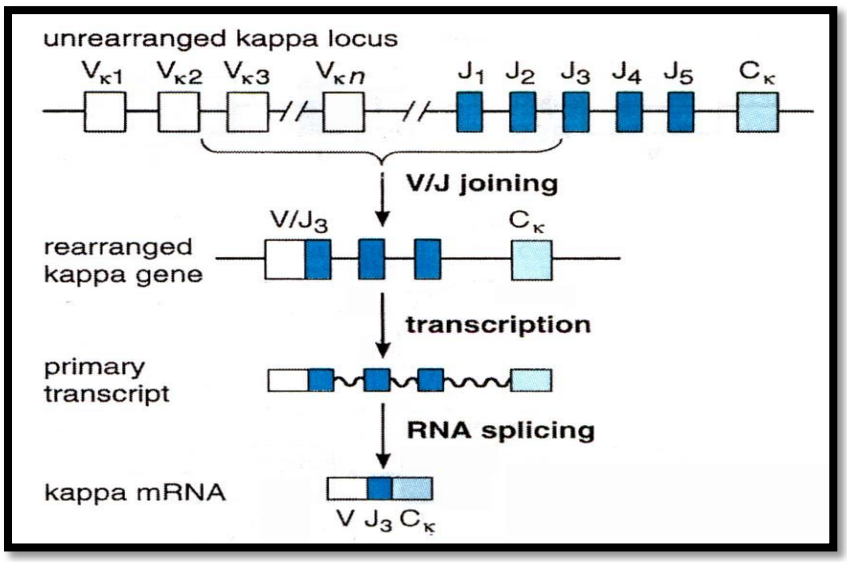
	B cell receptor (antibody, Ig)	T cell receptor (TCR)
		
		
Forms of antigens recognized	Macromolecules (proteins, polysaccharides, lipids, nucleic acids), small chemicals Conformational and linear epitopes	Mainly peptides displayed by MHC molecules on APCs Linear epitopes
Diversity	Each clone has a unique specificity; potential for $>10^9$ distinct specificities	Each clone has a unique specificity; potential for $>10^{11}$ distinct specificities
Antigen recognition is mediated by:	Variable (V) regions of heavy and light chains of membrane Ig	Variable (V) regions of α and β chains of the TCR
Signaling functions are mediated by:	Proteins (Igα and Igβ) associated with membrane Ig	Proteins (CD3 and ζ) associated with the TCR
Effector functions are mediated by:	Constant (C) regions of secreted Ig	TCR does not perform effector functions

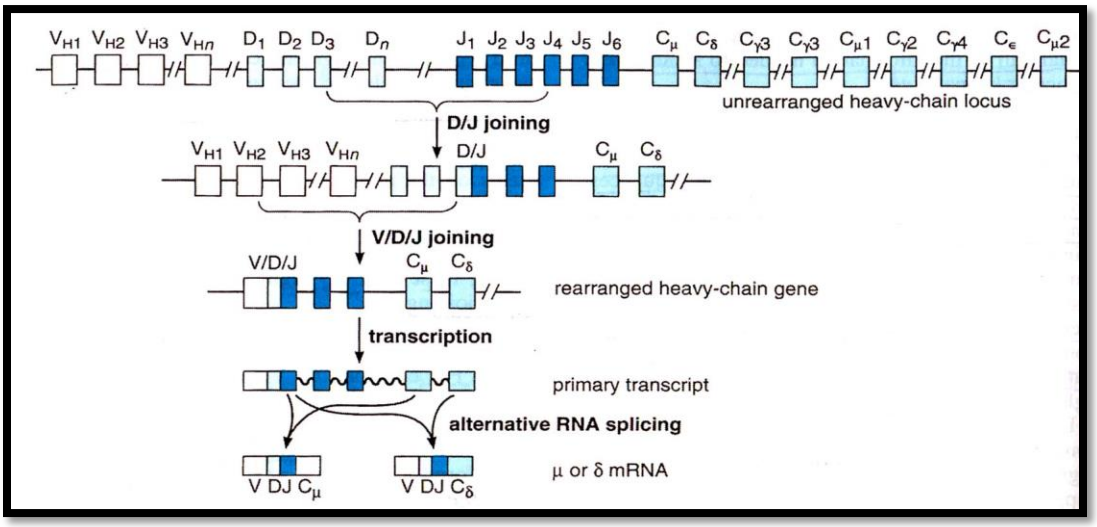
Development of immune repertoire (Diversity)

- The chromosomes contain no Ig or TCR genes but rather separated blocks or segments from which these genes can be assembled during the process of lymphocyte development.
- The process of gene assembly involves cutting, rearrangement, and rejoining of selected segments that encode V regions.
- V regions of the light chain of Ig and α chain of TCR are encoded by variable numbers of V and J segments on chromosome 14 & 2 respectively.
- ***V regions of the heavy chain of Ig and β chain of TCR are encoded by variable numbers of V, D & J segments on chromosome 14 & 7 respectively.***
- Differences in the constant (C) region of the heavy chain of Ig determine its Isotype.
- The C region is encoded on chromosome 14 by 9 segments which are corresponding to Ig isotypes & sub classes MAGED.
- 4 gamma segments: IgG 1,2,3,4.
  - ✓ 2 alpha segments : IgA 1,2.
  - ✓ One MU segments: IgM.
  - ✓ One Delta segments: for IgD.
  - ✓ One Epsilon segments: for IgE.

<u>Combinatorial diversity:</u>

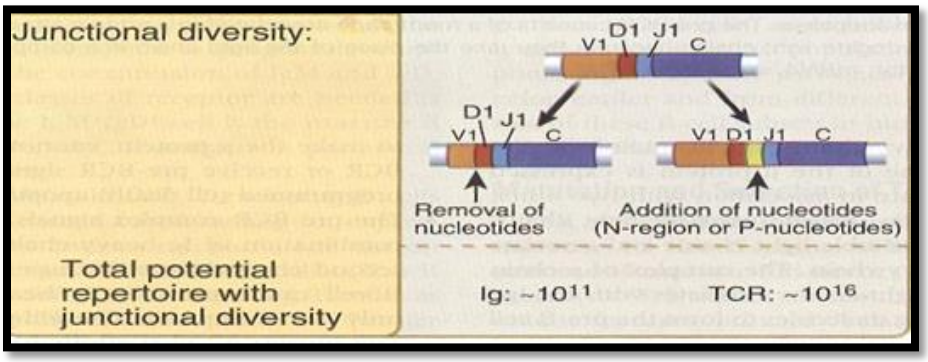
- It is the result of use of different combinations of V, D, J gene segments.
- Somatic combinations of V& J or VD & J segments are mediated by a group of enzymes.
  1- VDJ recombinases (RAG-1 &RAG-2) that recognize and cleave DNA specifically at a recombinational signal sequence (RSS).
  2- Artmis is an endonuclease that opens up at the coding ends.
  3- DNA- dependent protein kinase (DNA-PK) is a repair enzyme, DNA ligase.





Junctional diversity

- Results from changes in nucleotide sequence at the junctions of V, D & J segments in 3 ways.
  1- Removal of some nucleotides from V, D or J segment at time of recombination by "exonucleases".
  2- Addition of nucleotides that are not part of germ line genes to V,D or J segments forming N region, this is carried by the enzyme, terminal deoxy ribonucleotidyl transferase (TDT).
  3- Overhanging DNA sequences before completing recombination process forming "p-nucleotides".



Maturation and selection of lymphocytes

Steps:

1. Commitment of hematopoietic progenitors to B cell or T cell lineage
2. Proliferation of developing lymphocytes to ensure that an adequate number of cells will be available to express useful antigen receptors this occurs mainly under the influence of IL-7.
3. Activation of several lineages– specific transcription factors and increased accessibility of Ig & TCR genes for the process of re-arrangement & recombination.
4. The rearrangement & expression of antigen receptor genes.
5. Selection events
   - ✓ Positive towards useful receptors.
   - ✓ Negative against strong recognition of self.

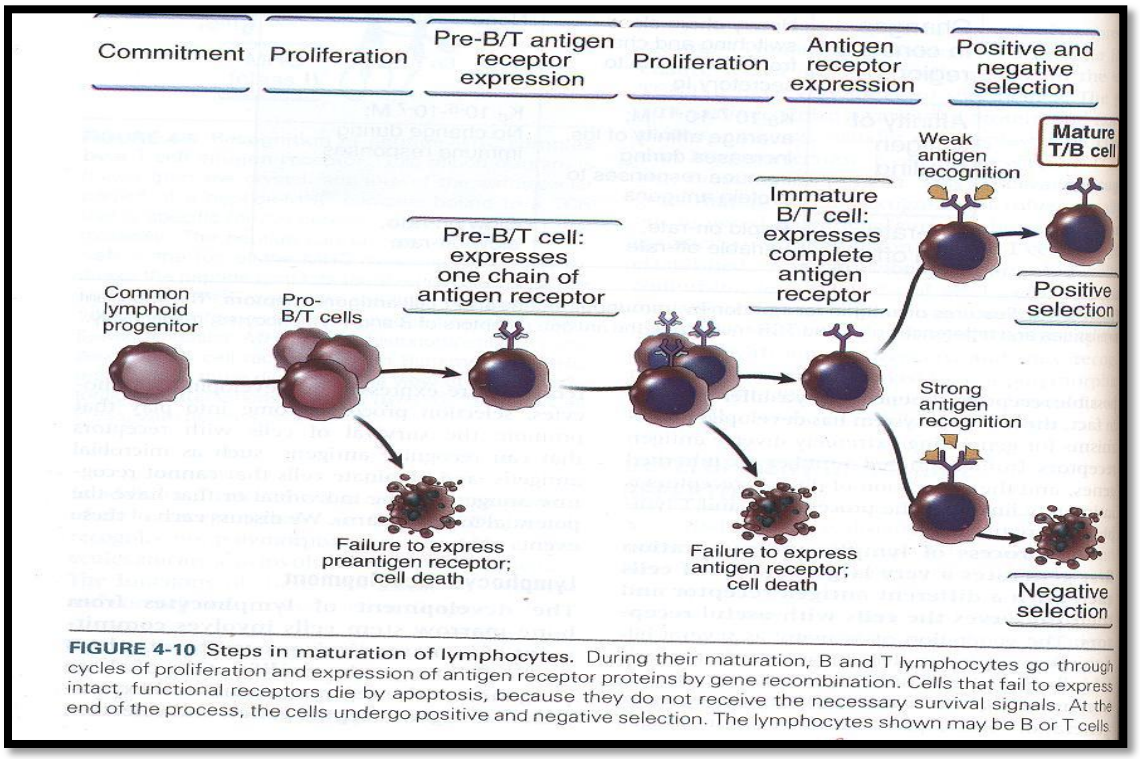


FIGURE 4-10 Steps in maturation of lymphocytes. During their maturation, B and T lymphocytes go through cycles of proliferation and expression of antigen receptor proteins by gene recombination. Cells that fail to express intact, functional receptors die by apoptosis, because they do not receive the necessary survival signals. At the end of the process, the cells undergo positive and negative selection. The lymphocytes shown may be B or T cells

Maturation & selection of B- lymphocytes

- Starts & ends in bone marrow. Some may complete maturation in the spleen.
- Pro – Pre-B –immature B – mature B.

- **Pro B cells: Proliferation and begin to rearrange the heavy chain locus.**

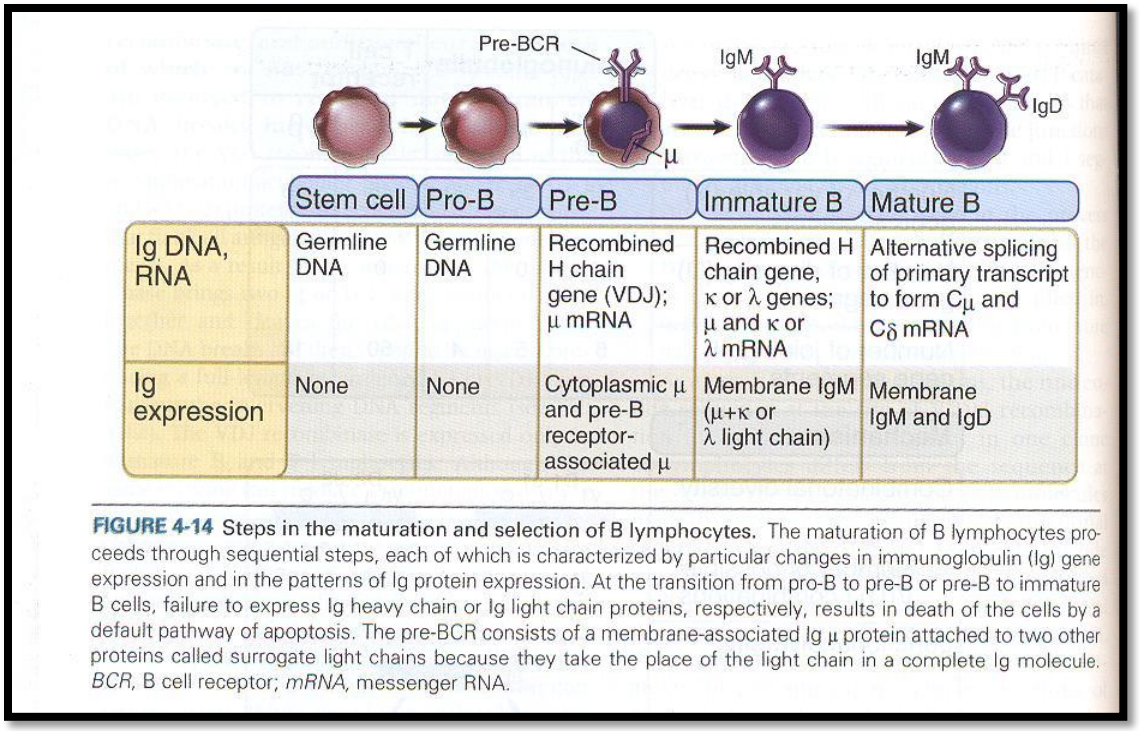
- **Pre B cells: Successfully made *Pre-BCR* which is composed of:**
  1- µ heavy chain protein
  2- Surrogate light chain
  3- Igα and Igβ

    - This promotes:
    - ✓ Delivery of signals from the surrounding through the Pre BcR: promotes ***survival &* proliferation** of these cells.
    - ✓ Allelic exclusion: The other chromosome will not make another heavy chain.
    - ✓ Triggering synthesis of κ light chain.
    - If the expressed Pre- BCR is none functioning: ***death by neglect "apoptosis"***

- **Immature B: Successfully has BCR composed of:**
  - ✓ µ Heavy chain, light chain and Igα and Igβ
  - ✓ The receptor again delivers signals that promotes survival
  - ✓ If the receptor shows high affinity binding to antigens present in the bone marrow the cell may be:
    a- Activates the VDJ recombinase enzyme to generate a different light chain , so changing the specificity of Ag receptor, this is called ***receptor editing*** or undergo
    b- ***Negative selection*** by apoptosis.

- **Mature B lymphocytes:**
  - ✓ The final maturation necessitates CO expression of IgD molecule with IgM
  - ✓ Most mature B cells are called follicular
  - ✓ Marginal zone B cells develop also from the same precursors while B1 lymphocytes develop from different precursors.

N.B: Self- molecules expressed in BM are plasma proteins, extracellular matrix proteins and surface molecules common to all cells.



	Stem cell	Pro-B	Pre-B	Immature B	Mature B
Ig DNA, RNA	Germline DNA	Germline DNA	Recombined H chain gene (VDJ); μ mRNA	Recombined H chain gene, κ or λ genes; μ and κ or λ mRNA	Alternative splicing of primary transcript to form C_μ and C_δ mRNA
Ig expression	None	None	Cytoplasmic μ and pre-B receptor-associated μ	Membrane IgM (μ+κ or λ light chain)	Membrane IgM and IgD

FIGURE 4-14 Steps in the maturation and selection of B lymphocytes. The maturation of B lymphocytes proceeds through sequential steps, each of which is characterized by particular changes in immunoglobulin (Ig) gene expression and in the patterns of Ig protein expression. At the transition from pro-B to pre-B or pre-B to immature B cells, failure to express Ig heavy chain or Ig light chain proteins, respectively, results in death of the cells by a default pathway of apoptosis. The pre-BCR consists of a membrane-associated Ig μ protein attached to two other proteins called surrogate light chains because they take the place of the light chain in a complete Ig molecule. *BCR*, B cell receptor; *mRNA*, messenger RNA.

Maturation and selection of T –lymphocytes

- T cell progenitors migrate from bone marrow to the thymus where the entire process of maturation occurs.
- Pro T cells – Pre T cell - immature T cell – mature T cell.

- **Pro T cells (double negative):** proliferation under the effect of IL-7 and begin to rearrange the β Chain locus.

- **Pre T cells:** expressing a pre TCR on surface formed of:
  - ✓ β- Chain
  - ✓ Pre –T α (invariant protein)
  - ✓ CD3 complex.
    - This promotes:
    1. Delivery signals through the Pre TCR: promotes survival and proliferation.
    2. Allelic exclusion.
    3. Triggering synthesis of α chain.
    - If the expressed pre-TCR is non-functioning: Death by neglect "apoptosis"

- **Immature T " Double positive T lymphocytes**: CD4+, CD8+ molecules
  - ✓ Now are exposed to the process of selection.
    1- Immature double positive T cells whose receptors strongly recognize MHC- peptide complex: undergo apoptosis: negative selection or develop to T- regulatory.
    2- Immature double positive T cells whose receptors don't recognize an MHC- peptide : die by apoptosis " death by neglect"
    3- Immature double positive T cells whose receptors recognize MHC–peptide complex with low or mild affinity: positively selected to survive and functionally segregated to be either
    CD4 + helper: T cell that recognizes the peptide with MHC class II.
    CD 8+ cytotoxic: T cell that recognizes the peptide with MHC class I.

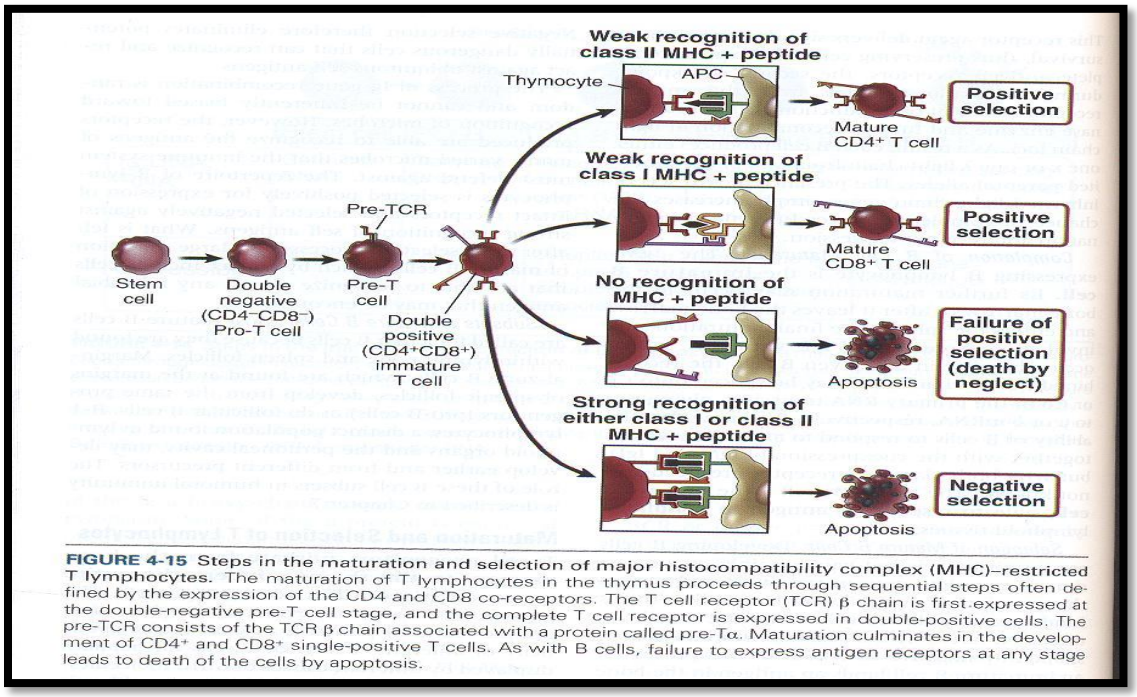


FIGURE 4-15 Steps in the maturation and selection of major histocompatibility complex (MHC)–restricted T lymphocytes. The maturation of T lymphocytes in the thymus proceeds through sequential steps often defined by the expression of the CD4 and CD8 co-receptors. The T cell receptor (TCR) β chain is first expressed at the double-negative pre-T cell stage, and the complete T cell receptor is expressed in double-positive cells. The pre-TCR consists of the TCR β chain associated with a protein called pre-Tα. Maturation culminates in the development of CD4+ and CD8+ single-positive T cells. As with B cells, failure to express antigen receptors at any stage leads to death of the cells by apoptosis.

The process of maturation occurs under the influence of many transcription factors:

- **GATA3** ***Notch 1***
- **Fork head box N1 (Fox N1):** needed for thymic organogenesis and attraction of hematopoietic cells to the thymus.
- **FOXP3:** necessary for T-reg function. Mutation leads to IPEX syndrome: immune dysregulation polyendocrinopathy enteropathy x linked characterized by T cells attack on multiple organs and autoantibodies production.

- **Autoimmune regulator (AIRE):** transcription factor responsible for expression of organ specific non-thymic peptides. Mutation of AIRE will lead to autoimmune polyendocrinopathy syndrome.

During development of lymphocytes "T or B" many nuclear events are regulated by epigenetic mechanisms.

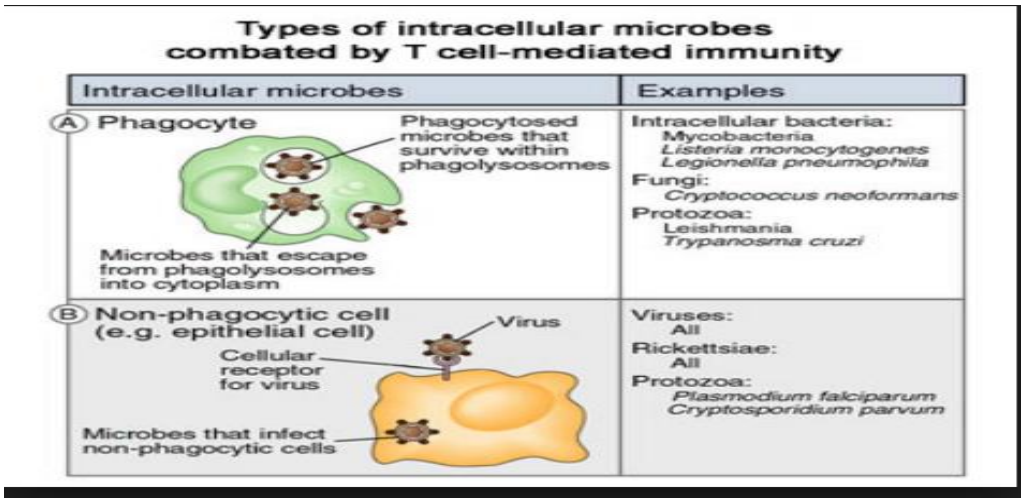
Epigenetics: The mechanism that controls gene expression that goes beyond the actual sequence of DNA in individual genes.
e.g.: the mechanisms that make genes available or unavailable in chromatin for transcription.
1- Methylation of DNA: generally silences genes.
2- Post transitional modification of histone: acetylation –methylation, ubiquitination which may render genes active or inactive.
3- Remodeling complexes.
4- Micro – RNA" mi RNA": e. g: mutation in Dicer: preferential loss of T-reg and in B lineage it results in a block at the Pro B to Pre B transition.

Cell mediated immunity

Definition:

- The form of adaptive immunity that is mediated by T lymphocytes.
- Cell mediated immunity is mainly responsible to combat the intracellular microbes that:

1- Escape the mechanism of killing inside phagocytic cells "Mycobacteria, Listeria, protozoa and fungi.
2- Infect non phagocyte cells, live & replicate into cytoplasm e.g.: viruses



- It also serves a defense mechanism against extracellular fungi and helminthic parasites.

- Steps:

1- Antigen recognition
2- T cell activation and proliferation
3- T cell differentiation to effectors & their responses.

Antigen recognition

- Accomplished via presentation by a professional antigen presenting cell which is the dendritic cell, the only cell capable of presenting antigen and activating naïve T cell.
- Dendritic cells in various tissues are capable of capturing antigens "microbes" that pass through epithelia (or enter the spleen) then migrate to the nearest regional LN through the afferent lymphatic vessels.

- During migration
    1- They lose adhesiveness to tissues first.
    2- Start to express CCR_7 chemokines receptors.
    3- Express the co-stimulatory molecules B7.1, B7.2.
    4- Express the adhesion molecule ICAM -1.

- The antigen inside the dendritic cell will be processed & loaded over an MHC molecule before expressed on the surface to be presented to a T- lymphocyte in either 2 ways.

1- ***For vesicular antigens*** : for microbes endocytosed into phagosomes that fuse with lysosomes, the antigen will be processed into peptides that will be loaded onto MHC- Class II molecules to be presented to CD4 " helper T cells" this is called class II MHC pathway.

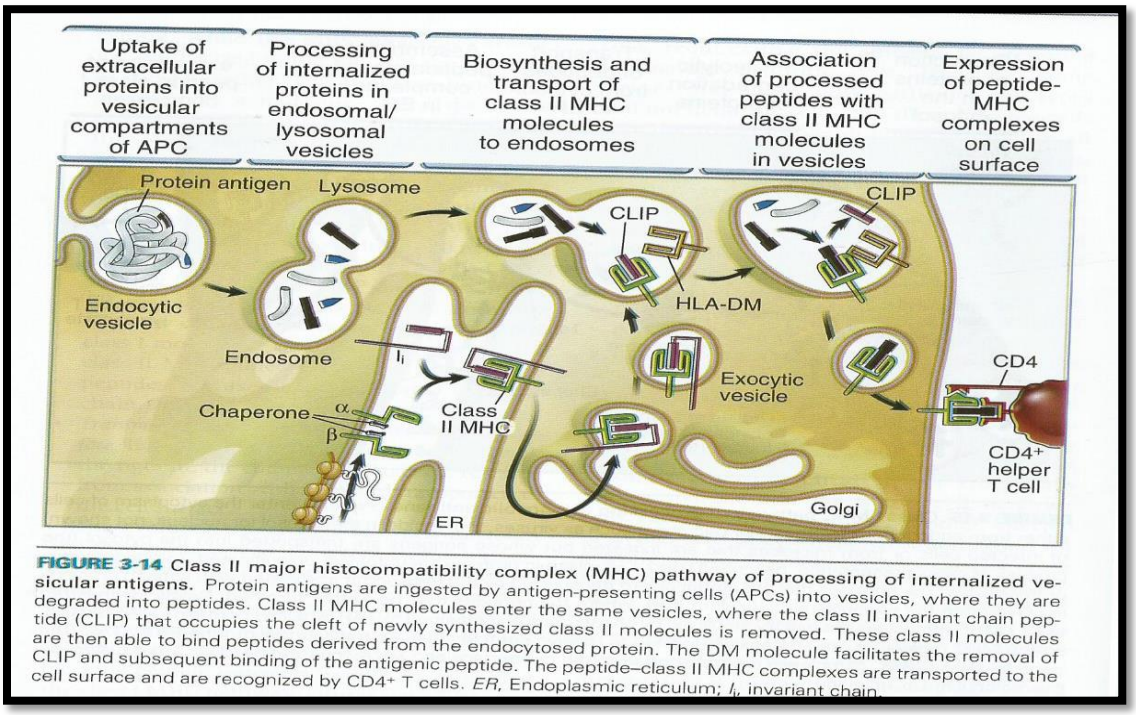


FIGURE 3-14 Class II major histocompatibility complex (MHC) pathway of processing of internalized vesicular antigens. Protein antigens are ingested by antigen-presenting cells (APCs) into vesicles, where they are degraded into peptides. Class II MHC molecules enter the same vesicles, where the class II invariant chain peptide (CLIP) that occupies the cleft of newly synthesized class II molecules is removed. These class II molecules are then able to bind peptides derived from the endocytosed protein. The DM molecule facilitates the removal of CLIP and subsequent binding of the antigenic peptide. The peptide–class II MHC complexes are transported to the cell surface and are recognized by CD4+ T cells. *ER*, Endoplasmic reticulum; *I_i*, invariant chain.

2- ***For cytosolic antigens***: microbes present in the cytoplasmic compartment e.g: viruses will be processed and loaded on MHC class I molecules and expressed extracellulary to be presented to CD8+ cytotoxic cell. This is called class I MHC pathway.

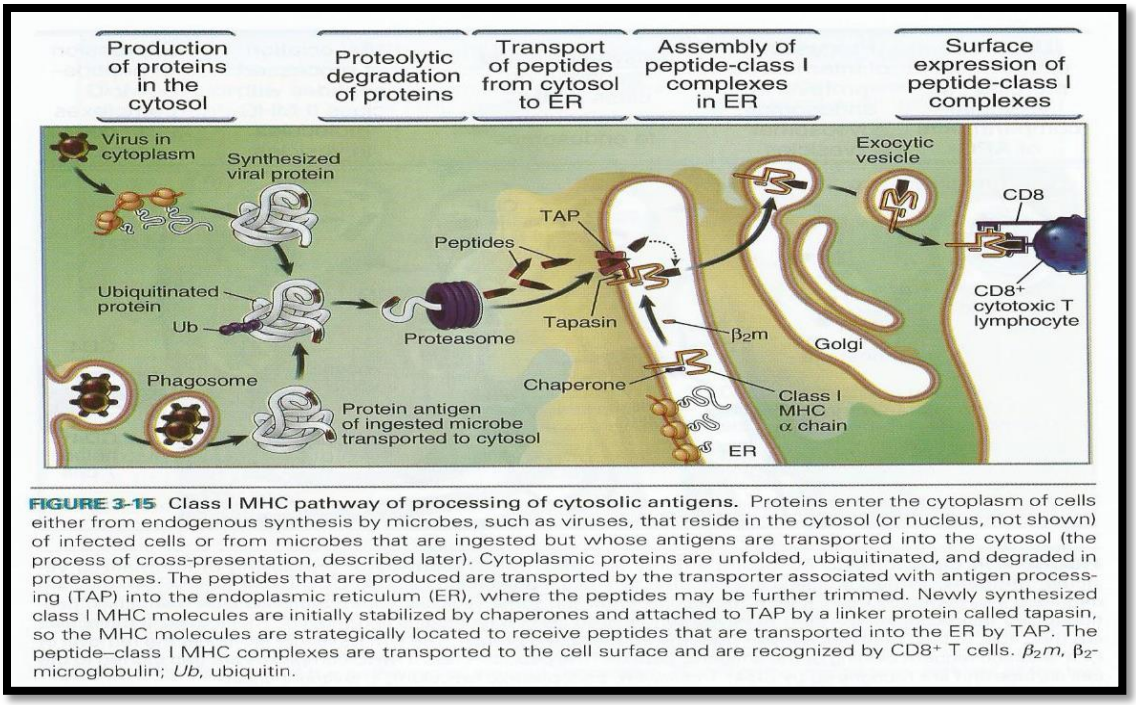


FIGURE 3-15 **Class I MHC pathway of processing of cytosolic antigens.** Proteins enter the cytoplasm of cells either from endogenous synthesis by microbes, such as viruses, that reside in the cytosol (or nucleus, not shown) of infected cells or from microbes that are ingested but whose antigens are transported into the cytosol (the process of cross-presentation, described later). Cytoplasmic proteins are unfolded, ubiquitinated, and degraded in proteasomes. The peptides that are produced are transported by the transporter associated with antigen processing (TAP) into the endoplasmic reticulum (ER), where the peptides may be further trimmed. Newly synthesized class I MHC molecules are initially stabilized by chaperones and attached to TAP by a linker protein called tapasin, so the MHC molecules are strategically located to receive peptides that are transported into the ER by TAP. The peptide–class I MHC complexes are transported to the cell surface and are recognized by $CD8^+$ T cells. β_2m, β_2-microglobulin; *Ub*, ubiquitin.

T cell activation

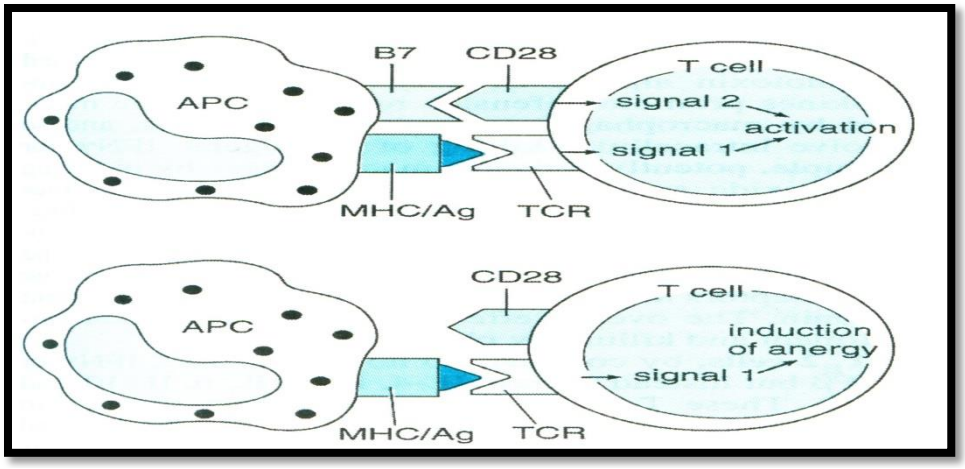
- Initiation of T cell activation occurs in the T cell zone of 2ry lymphoid organs and requires

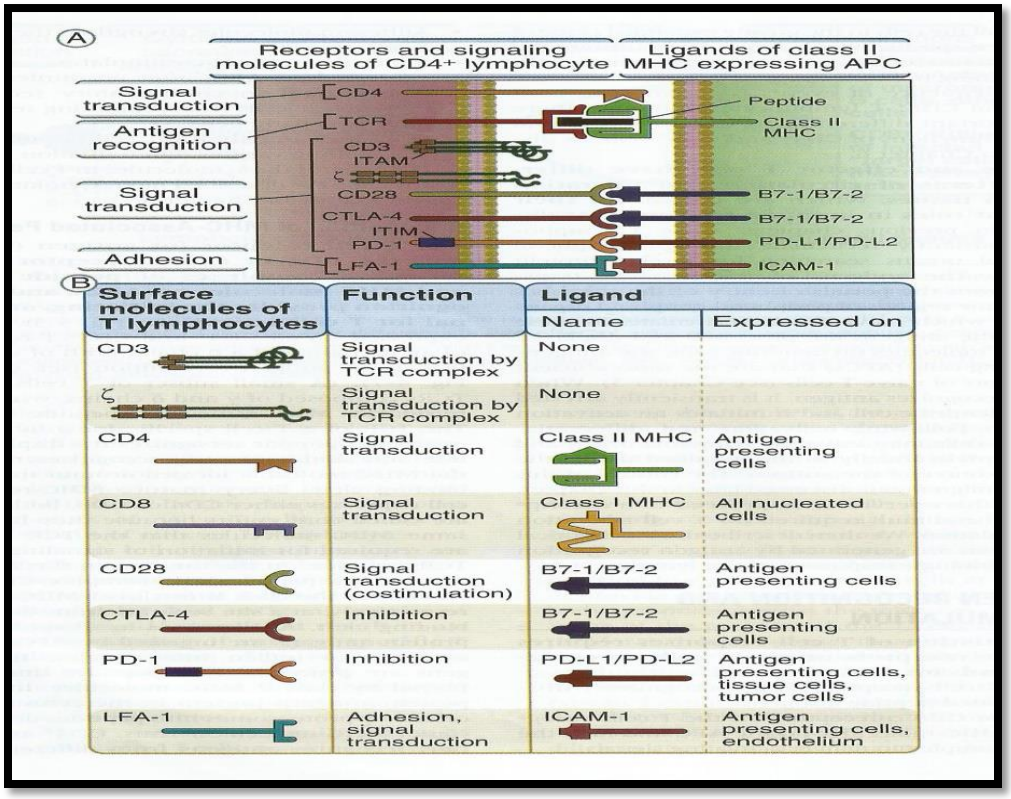
Signal I: TCR recognizes Ag presented with MHC molecules.

- ✓ Co-receptors: CD4 or CD8 recognizes domain of MHC molecule.
- ✓ Adhesion molecules to strengthen the binding of ICAM-1 on APC with LFA-1 on T cell.

Signal II: Offered by co-stimulators.

- ✓ B7.1 & B7.2 "CD80, CD86" on APC with CD28 on T cell.
- ✓ Other members of CD28 family and those of B7 ligands have been identified, they regulate T cell function either +ve or –ve.





Surface molecules of T lymphocytes	Function	Ligand	
		Name	Expressed on
CD3	Signal transduction by TCR complex	None	
ζ	Signal transduction by TCR complex	None	
CD4	Signal transduction	Class II MHC	Antigen presenting cells
CD8	Signal transduction	Class I MHC	All nucleated cells
CD28	Signal transduction (costimulation)	B7-1/B7-2	Antigen presenting cells
CTLA-4	Inhibition	B7-1/B7-2	Antigen presenting cells
PD-1	Inhibition	PD-L1/PD-L2	Antigen presenting cells, tissue cells, tumor cells
LFA-1	Adhesion, signal transduction	ICAM-1	Antigen presenting cells, endothelium

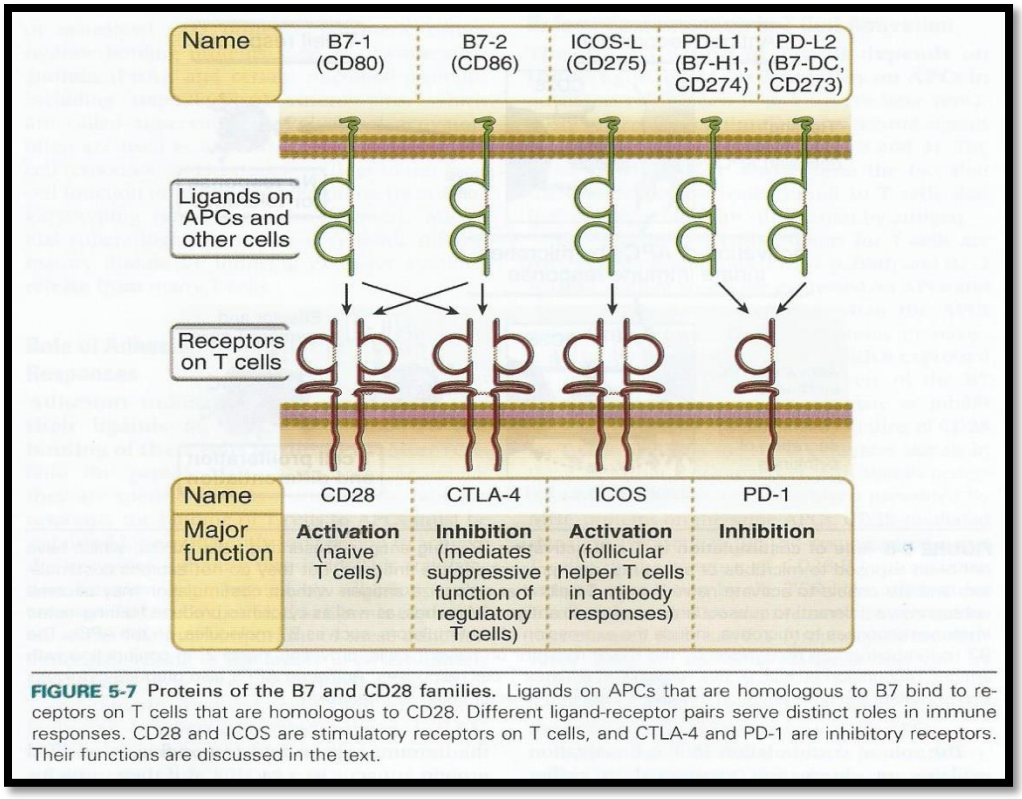


FIGURE 5-7 Proteins of the B7 and CD28 families. Ligands on APCs that are homologous to B7 bind to receptors on T cells that are homologous to CD28. Different ligand-receptor pairs serve distinct roles in immune responses. CD28 and ICOS are stimulatory receptors on T cells, and CTLA-4 and PD-1 are inhibitory receptors. Their functions are discussed in the text.

Biochemical pathways of T cell activation.

- After Ag recognition and full activation of the T-cell. Intracellular biochemical pathways all consists of:
  1- ***Activation of enzymes***
  2- ***Recruitment of adaptor proteins***
  3- ***Activation of transcription factors.***

- The changes start by phosphorylation of the tyrosine motifs present in ITAM "immune receptor tyrosine based activation motifs" present in the signaling molecule as CD3 complex.

- LCK is a protein tyrosin kinase that is none covalently attached to cytoplasmic tail of the CO-receptor CD4& CD8. LCK will phosphorylate the ITAM motifs → Opening of a docking site in it from another tyrosin kinase called “Zap 70” which then activated by LCK and leads to phosphorylation of various adaptor proteins & enzymes that mediate the additional signaling events.

- Now the major 4 signaling pathways will continue as follows:

1- <u>Nuclear factor of activated T cells (NFAT) pathway</u>

- ✓ Zap 70 phosphorylates & activates enzyme called phospholipase CY (PLCY) which catalyze the hydrolysis of the plasma membrane phospholipid called phosphatidyl inositol 4,5 biphosphonate (PIP2) to inositol 1,4,5 triphosphate (IP3) and diacylglycerol (DAG)
- ✓ IP3 will stimulate release of Ca from ER → Opening of a plasma membrane Ca channel → Influx of extracellular Ca → Sustained elevation of Ca → Binding and activation of Calmodulin → CA calmodulin complex → Activation of phosphatase called calcineurin which will activate the transcription factor NFAT. This transcription factor will activate the expression of several genes including IL-2 & its receptor.
- ✓ ***Cyclosporin acts by binding to & inhibits the phosphates activity of Calcineurin.***

2- <u>The nuclear factor-κβ (NFκβ) pathway:</u>

- ✓ DAG released from the membrane phospholipid will activate protein kinase C (PKC)
- ✓ PKC acts through adaptor proteins recruited to TCR complex to activate the NFκβ which will promote the transcription of several genes.

3- <u>The RAS/RAC –MAP kinase pathway</u>

- ✓ The active Ras GTP/Rac GTP→ Activation of mitogen activated protein (MAP) kinases. The terminal MAP kinases in these pathways are:
  - ERK" Extracellular signal – related kinases and JNK "june amino terminal kinase". These 2 kinases will activate the c-fos & c-jus.
  - c-fos & c-jus combine to form the transcription factor AP-1 "activating protein -1" that promotes the transcription of various T Cell genes.

4- <u>Phosphatidyl inositol-3 (IP-3) pathway</u>

- ✓ Which phosphorylates membrane PIP2 to PIP3.
- ✓ PIP3 is needed for activation of a serine-threonine kinase called protein kinase or AKt → Expression of anti-apoptotic proteins thus promoting survival of the Ag–stimulated T cells.

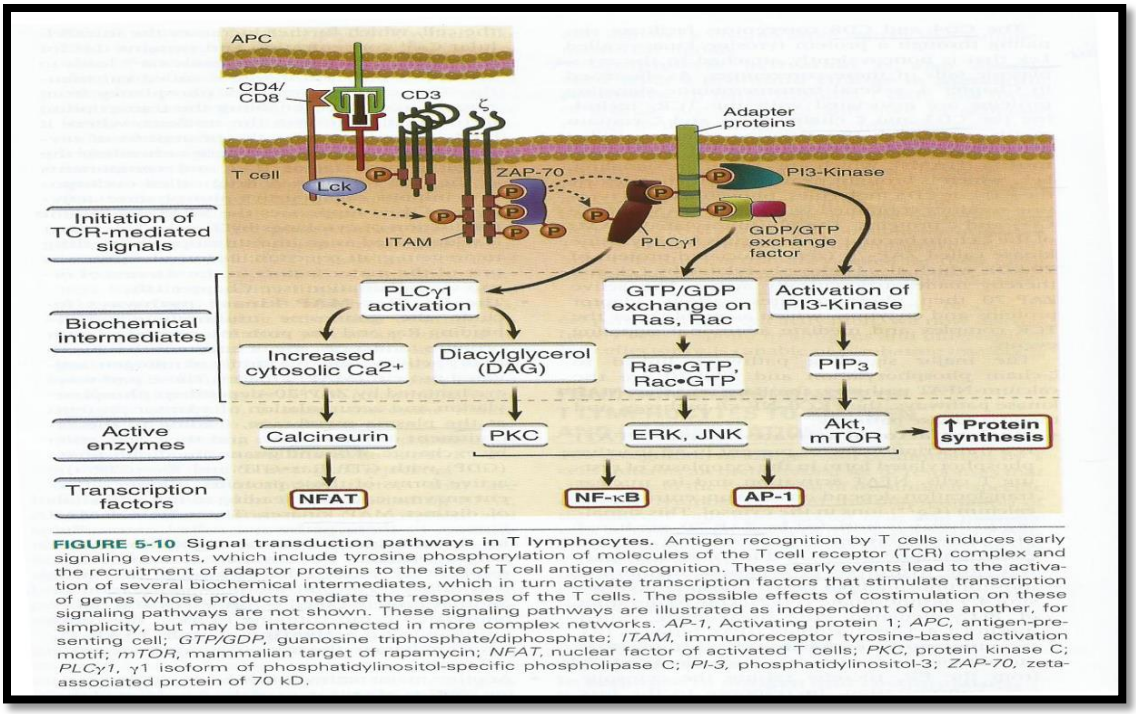


FIGURE 5-10 Signal transduction pathways in T lymphocytes. Antigen recognition by T cells induces early signaling events, which include tyrosine phosphorylation of molecules of the T cell receptor (TCR) complex and the recruitment of adaptor proteins to the site of T cell antigen recognition. These early events lead to the activation of several biochemical intermediates, which in turn activate transcription factors that stimulate transcription of genes whose products mediate the responses of the T cells. The possible effects of costimulation on these signaling pathways are not shown. These signaling pathways are illustrated as independent of one another, for simplicity, but may be interconnected in more complex networks. *AP-1*, Activating protein 1; *APC*, antigen-presenting cell; *GTP/GDP*, guanosine triphosphate/diphosphate; *ITAM*, immunoreceptor tyrosine-based activation motif; *mTOR*, mammalian target of rapamycin; *NFAT*, nuclear factor of activated T cells; *PKC*, protein kinase C; *PLCγ1*, γ1 isoform of phosphatidylinositol-specific phospholipase C; *PI-3*, phosphatidylinositol-3; *ZAP-70*, zeta-associated protein of 70 kD.

The Functional sequence of T cell activation is

1- Secretion of cytokines and expression of cytokine receptors.
2- Proliferation of the cells expressing the same TCR that recognizes the Ag this is called clonal expansion.
   The magnitude of clonal expansion is more for CD8 than CD4.
3- Differentiation of Naïve T cells into effector cells with production of many cytokines. Cytokines of the adaptive immune system are characterized by pleotropism and redundancy.

The first cytokine secreted by activated CD4 cells is IL-2 “within 1-2 hours of Ag recognition" together with expression of high affinity IL-2 receptors.
IL-2 cytokine "previously known as **T-Cell growth factor"** will stimulate the survival and proliferation of the Ag- specific T cells and also maintain T-regulatory cell function which aid in homeostasis later on.

T cell differentiation to effector and memory cells

- The process of differentiation is the **result of gene expression s**uch as:
  - ✓ Activation of genes encoding cytokines" in CD4 T cell subsets"
  - ✓ Activation of genes encoding cytotoxic proteins "in CD8 CTLS"

- The mechanisms that determine whether a differentiated T cell will become a short lived effector cell or a long lived memory cell are not yet established. One possibility refers to the type of the transcription factors induced during the process of activation.
  - ✓ ***T- Bet is said to drive the differentiation to effector.***
  - ✓ ***Blimp-1 is said to drive the differentiation to memory cells***.

- The effector cells leave the 2ry lymphoid organs to the periphery and again encounter the Ag that stimulated then differentiation to start the effector responses. Other cells migrate to the lymphoid follicles to help B cells yet other cells "the most" stay as memory cells and known as central memory to be differentiated from effector memory cells present in the epithelium at the site of entry ready to fast react with re infection.

General features of T cell subset differentiation

1- Differentiation of Naïve CD4 T- lymphocytes to various subsets occurs mainly in ***response to cytokines*** present early during immune response and involves transcriptional activation and epigenetic modification of cytokine genes.
2- Cytokines produced by any given subset promote the development of this subset "amplification" and inhibit differentiation towards other subsets.
3- Source of cytokines that derive the development :
   - ✓ **APC: DC & macrophages.**
   - ✓ **NK cells, basophils & mast cells**.
4- Stimuli other than cytokines that influence the differentiation :
   - ✓ ***Dendritic subsets***
   - ✓ ***Genetic factors***
5- Differentiation of each subset is induced by ***the types of the microbes t***hat the subset is best able to combat. Thus ensuring specialization which is considered one of the important feature of the adaptive immune response.

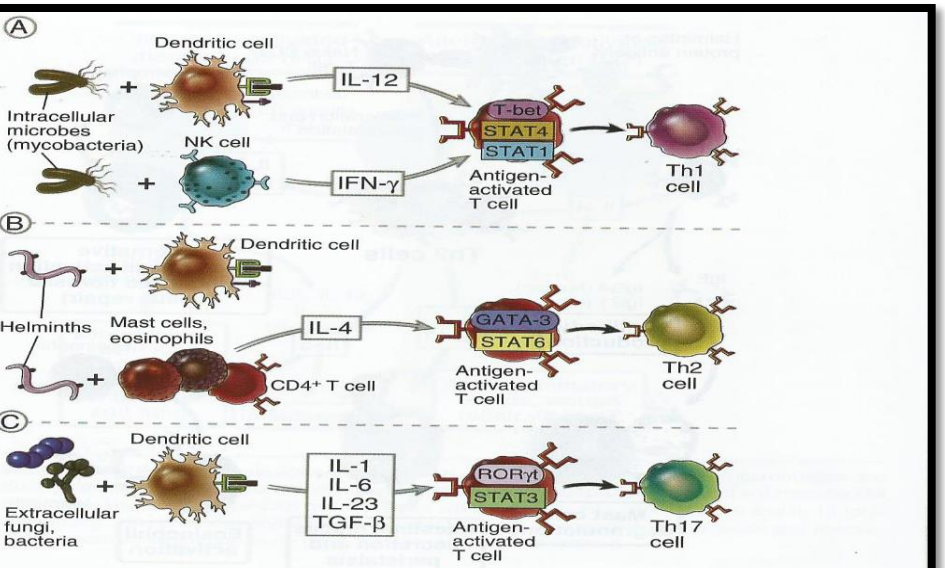


FIGURE 6-7 Development of Th1, Th2, and Th17 effector cells. Dendritic cells and other immune cells that respond to different types of microbes secrete cytokines that induce the development of antigen-activated CD4+ T cells into Th1 (**A**), Th2 (**B**), and Th17 (**C**) subsets. The transcription factors that are involved in T cell differentiation are indicated in boxes in the antigen-activated T cells.

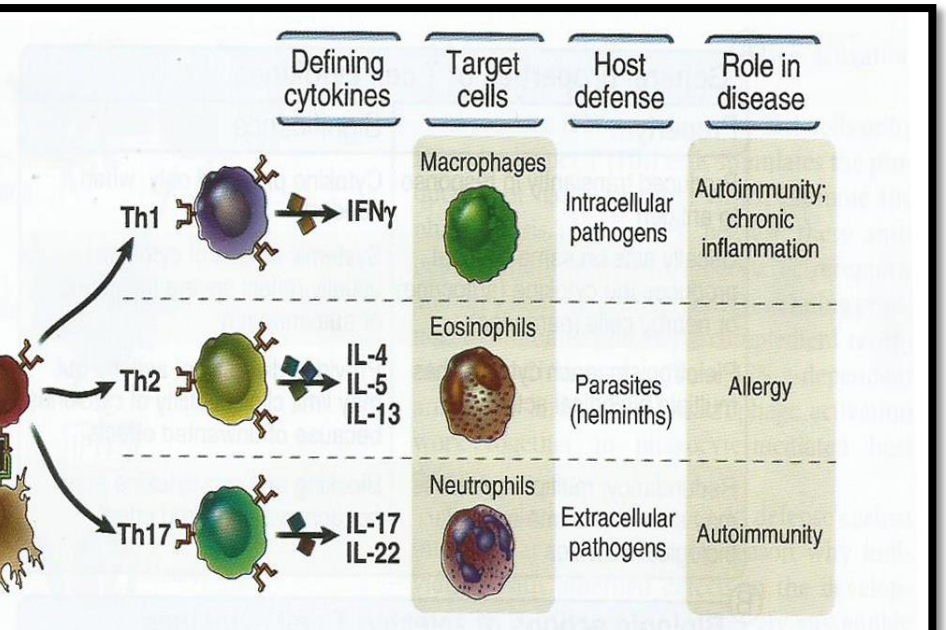


FIGURE 6-3 Characteristics of subsets of CD4+ helper T lymphocytes. A naive CD4+ T cell may differentiate into subsets that produce different cytokines that recruit and activate different cell types (referred to as *target cells*) and combat different types of infections in host defense. These subsets also are involved in various kinds of inflammatory diseases. The table summarizes the major differences among Th1, Th2, and Th17 subsets of helper T cells. *IFN*, Interferon; *IL*, interleukin.

T helper 1 subset differentiation and response

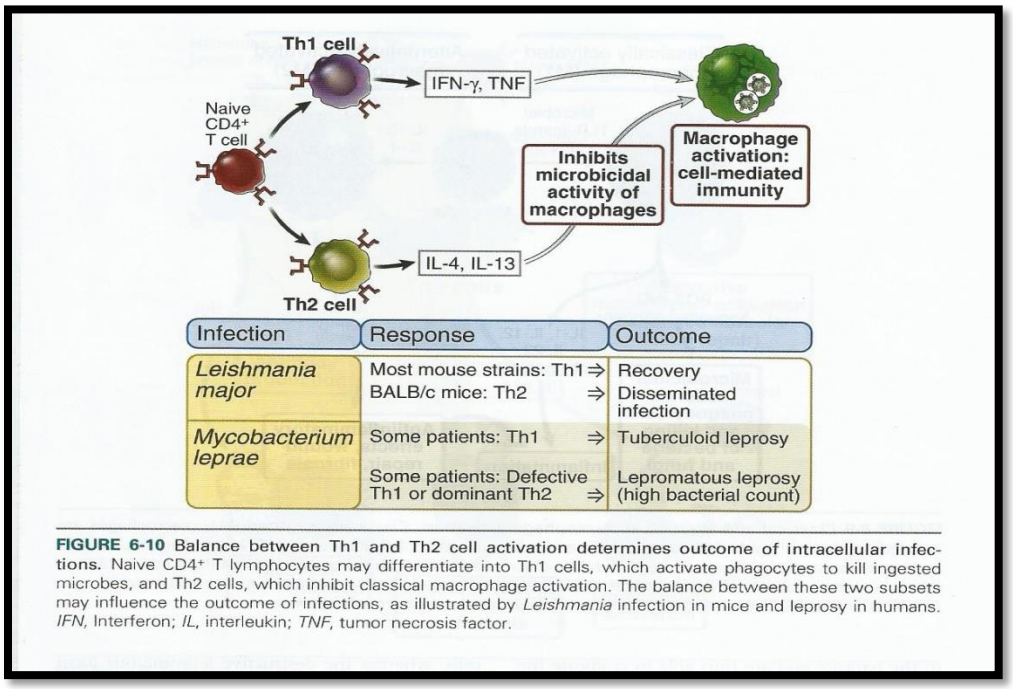
- **Induced by:** Many intracellular bacteria that are ingested by and activate phagocytes, DC, NK cells such as Listeria, mycobacteria & some parasites as lishmania.
- **Main cytokines needed:** IL-12& IFN-γ
- **Others:** IL-18 & Type 1 interferon.
- *The signature cytokine: INF- γ which further activates the differentiation*
- ***The transcription factors expressed in these subsets***:
  - ✓ T –Bet
  - ✓ STAT-1
  - ✓ STAT-4

Functions of T helper 1 cells

- The response mediated by this subset is called T cell stimulated, phagocyte – mediated killing of ingested microbes.
- Th1 cell secrete **IFN-γ** and express **CD40-L** molecules which interact with CD40 molecules on macrophages ingesting the same Ag that stimulate the differentiation of Th1.
- CD40-L when interacting with CD40 will activate the transcription factors NFκβ & AP-1. INF–γ activates STAT-1. These transcription factors will enhance the expression of genes encoding various enzymes in phagolysosomes namely → Phagocyte oxidase, NO synthase & lysozomal proteases that mediate the intracellular destruction of the microbes "***classic macrophage activation pathway"***. Toxic substances generated by these enzymes may be released → Tissue injury.
- Gene mutation in CD40-L or CD40 or both → "X–linked hyper IgM syndrome" Increased susceptibility to infection with otherwise harmless intracellular microbes.

- ***IFN-γ in addition to stimulation of classic activation of macrophage. It also :***
  1- Mediates isotype switching to IgG subclasses
  IgG will bind to Fc receptors on macrophage and activate complement: both actions lead to opsonization and phagocytosis of microbes.
  2- Inhibits Th2 & Th17 differentiation
  3- Stimulates expression of proteins involved in Ag processing & presentation by **APC & macrophage** → MHC molecules. TAP & HLA-DM and B7 co-stimulators → So macrophage become more potent at stimulating T cells → More secretion of IFN-γ → Positive feedback loop.

- Other cytokines secreted by Th1:
  - ✓ TNF & chemokines → Recruitment of leukocytes and enhancement of inflammation.
  - ✓ IL-10 which has suppressive actions on APC and macrophage → Inhibit Th1 thus representing an example of negative feedback loop in T cell response.



Infection	Response	Outcome
Leishmania major	Most mouse strains: Th1 ⇒	Recovery
	BALB/c mice: Th2 ⇒	Disseminated infection
Mycobacterium leprae	Some patients: Th1 ⇒	Tuberculoid leprosy
	Some patients: Defective Th1 or dominant Th2 ⇒	Lepromatous leprosy (high bacterial count)

FIGURE 6-10 Balance between Th1 and Th2 cell activation determines outcome of intracellular infections. Naive CD4+ T lymphocytes may differentiate into Th1 cells, which activate phagocytes to kill ingested microbes, and Th2 cells, which inhibit classical macrophage activation. The balance between these two subsets may influence the outcome of infections, as illustrated by *Leishmania* infection in mice and leprosy in humans. *IFN*, Interferon; *IL*, interleukin; *TNF*, tumor necrosis factor.

Th 2 subset: differentiation & response

- Th2 is the mediator of phagocyte-independent, eosinophil mediated immunity. These reactions are important in helminthic infections & allergy
- ***The main cytokine needed for differentiation: IL-4***
- Source of IL-4:
  - ✓ Tissue mast cell
  - ✓ Innate lymphoid cells
  - ✓ Th2 itself: secrete IL-4 → Positive feedback.
- The differentiation of this subset occurs in the absence of strong innate immune response.
- The signature cytokines: IL-4 mainly in addition to IL-5 & IL-13.
- The transcription factors expressed:
  - ✓ GATA-3
  - ✓ STAT-6

Functions of Th2

1- Isotype switching to IgE mainly by IL-4 → Binds to Fc receptors on mast cells and lead to degranulation.
2- Activation of eosinophils by IL-5 → Secrete toxic substances. IL-5 also triggers Isotype switching to IgA.
3- IL-13 → Induce alternative activation of macrophages → Tissue repair and fibrosis.
Stimulate mucus production.
4- Actions of IL-4 : development of Th2
   - ✓ Isotype switching to IgE
   - ✓ Stimulate intestinal peristalsis
   - ✓ Recruitment of eosinophils
   - ✓ Stimulate alternative macrophage pathway.

So the defense mechanisms offered by Th2 cells are accomplished through:

1- IgE & eosinophil mediated reactions
Binding of IgE will opsonize helminthic peptides to Fc receptors on eosinophils → Activation and release of eosinophils granular content which are toxic to the parasite.

2- Activation of mast cells
Again through binding of IgE to Fc receptors on mast cells will lead to degranulation and release of vasoactive amines.
In addition activated mast cells secrete → TNF- chemokines – lipid mediators
All lead to inflammation and destruction of parasite and allergic reactions.

3- **Barrier immunity:** through increased mucus secretions & increased intestinal peristalsis.

4- **Alternative macrophage pathway**: with production of IL-13 - TGFB & PDGF & fibroblast growth factor and resultant new blood vessel formation " angiogenesis", collagen synthesis, scarring and fibrosis.

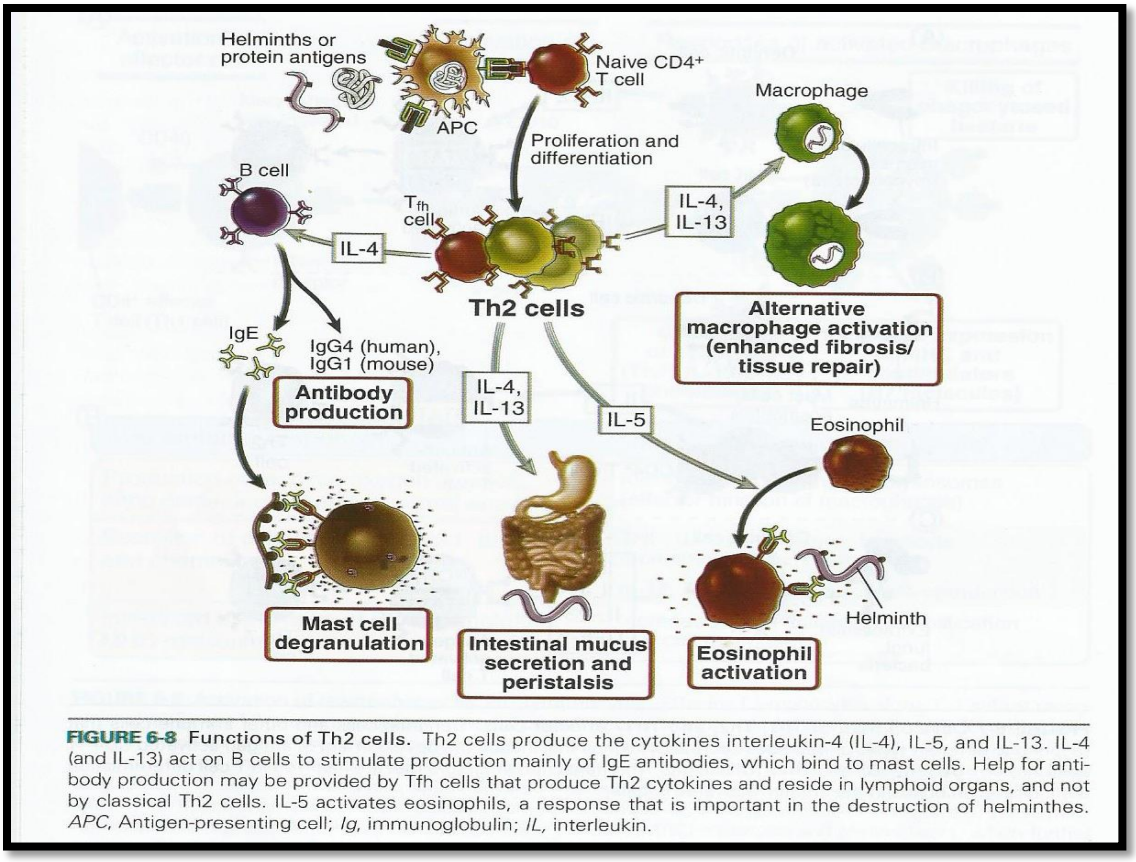


FIGURE 6-8 Functions of Th2 cells. Th2 cells produce the cytokines interleukin-4 (IL-4), IL-5, and IL-13. IL-4 (and IL-13) act on B cells to stimulate production mainly of IgE antibodies, which bind to mast cells. Help for antibody production may be provided by Tfh cells that produce Th2 cytokines and reside in lymphoid organs, and not by classical Th2 cells. IL-5 activates eosinophils, a response that is important in the destruction of helminthes. *APC*, Antigen-presenting cell; *Ig*, immunoglobulin; *IL*, interleukin.

Th17 subset differentiation & response.

- These cells are important in defense against extracellular & bacterial infection by recruiting leukocytes mainly neutrophils to sites of antigen recognition.
- ***Differentiation induced by cytokines:*** IL-6, IL-1, IL-23 & TGF-β
- ***The signature cytokines: IL-17 & IL-22***
- Transcription factors expressed: RORγt- STAT3
- Mutation in STAT3 will lead to ***JOB's syndrome "hyper IgE syndrome"*** characterized by increased susceptibility to cutaneous fungal & bacterial infection.

Functions of Th17

- *Mediated by IL-17 & IL-22*

✓ IL-17: unusual cytokine as the cytokine & its receptors are not homologous to any other known cytokine receptor pair

✓ It includes 6 structurally related proteins "A-F. A & F are the most similar & are responsible for the immune function sometimes called **immune inflammation**= strong acute inflammatory reactions that may accompany T cell responses.

IL-17

- ✓ **Induces neutrophil rich inflammation through increased production of TNF & chemokines.**
- ✓ **Stimulate the production of antimicrobial substances including defensins.**

IL-22

- ✓ **Maintain epithelial integrity by stimulating repair reaction.**
- ✓ **Stimulates chemokines production**.

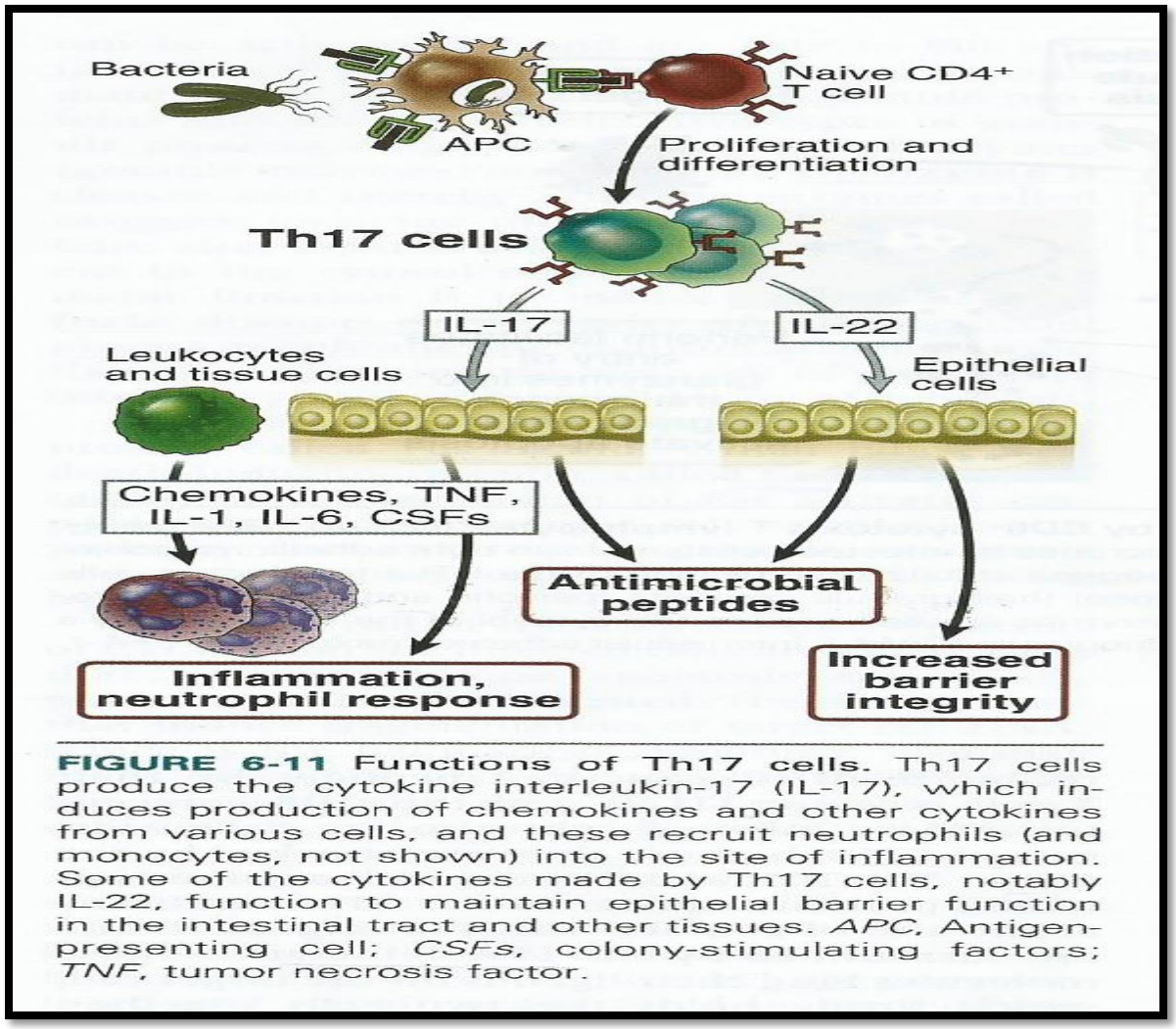


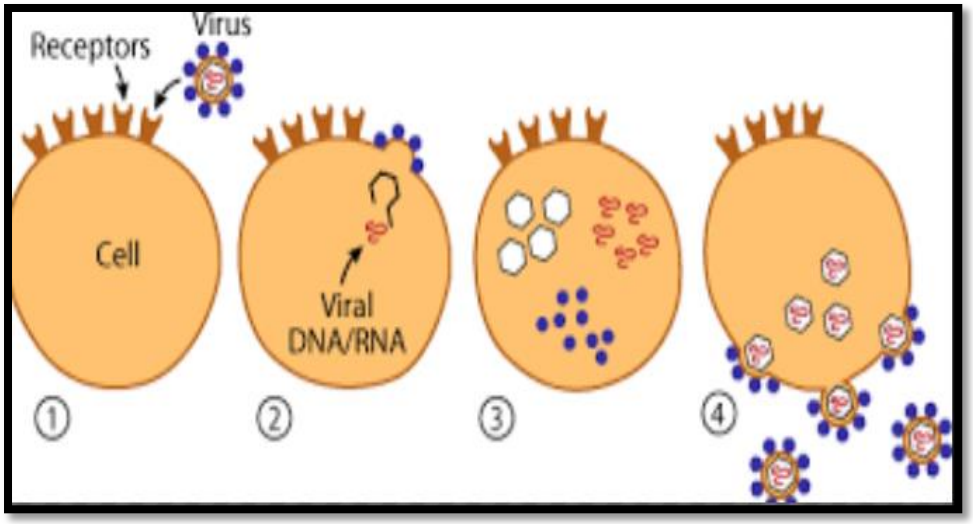
FIGURE 6-11 Functions of Th17 cells. Th17 cells produce the cytokine interleukin-17 (IL-17), which induces production of chemokines and other cytokines from various cells, and these recruit neutrophils (and monocytes, not shown) into the site of inflammation. Some of the cytokines made by Th17 cells, notably IL-22, function to maintain epithelial barrier function in the intestinal tract and other tissues. *APC*, Antigen-presenting cell; *CSFs*, colony-stimulating factors; *TNF*, tumor necrosis factor.

Differentiation & function of CD8 effector cells" CTLS"

Main function: killing of infected cells.

What happens with virus infection?

- ✓ The virus use the host cell's genetic and protein synthetic machinery to replicate and disseminate from cell to another.
- ✓ The virus cannot be destroyed if the infected cell lack the intrinsic microbicidal mechanisms.
- ✓ The virus cannot be destroyed if present in the cystosol compartment of the cell.
- ✓ Accordingly the only way to eradicate an established viral infection is to kill infected cells.



Activation of naïve CD8 needs:

1- **Antigen recognition.**
2- **Second signal**.

Antigen recognition:

- ✓ The CD8 cell recognizes Ag expressed with MHC Class I molecules. So classic APC is not suitable. alternatively a specialized Ag presenting cell will manage to present the virus to CD8 cell through a process known as cross presentation.

Cells responsible for cross presentation

1- **Dendritic cells** expressing CD141 also known as BDCA-3
2- **Plasmacytoid dendritic cells** "spleen"

Those cell engulf the virally infected tissue cells or viruses in the blood and transport the Ag of the infectious microbe to the cytosol to be processed and presented with class I to CD8 cells.

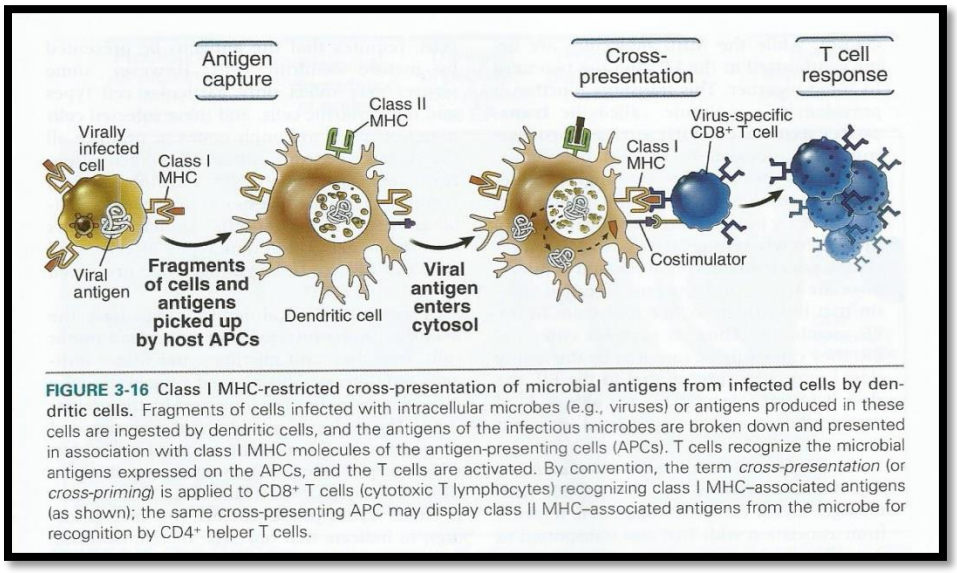


FIGURE 3-16 Class I MHC-restricted cross-presentation of microbial antigens from infected cells by dendritic cells. Fragments of cells infected with intracellular microbes (e.g., viruses) or antigens produced in these cells are ingested by dendritic cells, and the antigens of the infectious microbes are broken down and presented in association with class I MHC molecules of the antigen-presenting cells (APCs). T cells recognize the microbial antigens expressed on the APCs, and the T cells are activated. By convention, the term *cross-presentation* (or *cross-priming*) is applied to $CD8^+$ T cells (cytotoxic T lymphocytes) recognizing class I MHC–associated antigens (as shown); the same cross-presenting APC may display class II MHC–associated antigens from the microbe for recognition by $CD4^+$ helper T cells.

Second signal can be provided by CD4 helper cells through various mechanisms:

I- T-helper cytokines:
1-IL-2: proliferation & differentiation.
2-IL-12 & type 1 INF: differentiation.
3-IL-15: is important for survival of memory CD8
4-IL-21: is important for induction of memory CD8.
II- CD40L on activated T helper may bind CD40 on APC making them more efficient at stimulating CD8 in part by inducing expression of co-stimulators "B7.1. B7.2"

Mechanisms of killing by CTLS

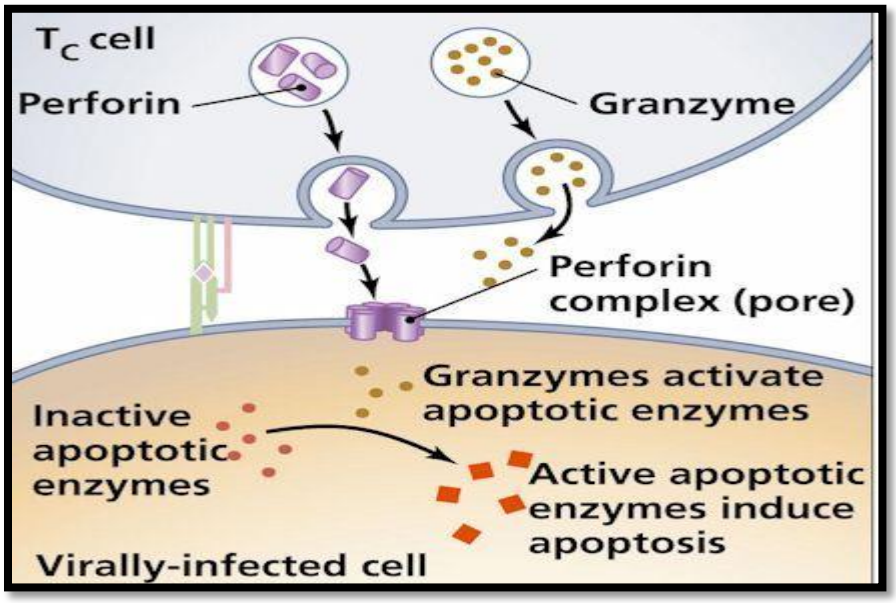
I-Recognition of target cells:

1. Through TCR recognizing Ag with Class I on the surface of infected cells
2. CO-receptors CD8.
3. Adhesion molecules"ICAM-1 to LFA-1"

II-Killing of target cells:

1. Secretion of cytotoxic granules present in the cytoplasm of CTLS:
   - ✓ *Perforins*: molecules homologous to cytotoxic granules which most probably lead to formation of aqueous pores in the plasma membrane of target cells facilitating introduction of granzymes.
   - ✓ *Granzymes*: will activate caspases and other substrates & initiate apoptotic death of target cells.

2. Activated CTLS also express FASL which bind FAS receptor on target cells → Death by apoptosis.



Homing & recruitment of T-lymphocytes

1- Homing to T-cell zone in secondary lymphoid organs.

i. Selectin mediated rolling:
   - ✓ L-Selectin on naïve T-lymphocyte with Selectin Ligand on endothelial cell of HEV.

ii. Integrin mediated firm adhesion:
   - ✓ LFA-1 on T-lymphocyte with ICAM-1 on endothelial cell.

iii. Transendothelial migration along concentration gradient of chemokines CCL19 & CCL21 produced in the T-cell zone "previously mentioned" for which the T-Lymphocyte express in CCR7 receptors.

2- Circulation between lymph nodes " searching for Ags"
   - ✓ In the LN t- cells ↑ the expression of the receptor for sphingosine 1-phosphate (S1P). As the concentration of S1P in blood & lymph is higher than inside the node so T- lymphocyte leave the node to the lymph then to the blood along concentration gradient.

3- On encountering Ag inside the LN:
   - ✓ Surface expression of S1P receptors s suppressed for several days "till the end of differentiation".

4- At the end of the process of differentiation→ ↑expression of SIP receptors & ↓ or loose of expression of CCR7 and L-Selectin → the activated T-cells are drawn out of the node.

5- Migration of effector T-cells to site of infection " same as neutrophils, previously mentioned in innate response"
   - ✓ Selectin mediated rolling:
     E & P selectin Ligands ↔ E & P selectin on endothelial cells.
   - ✓ Integrin mediated firm adhesion:
     LFA-1& VLA-4 on T-cells ↔ ICAM-1 & VCAM on endothelial cells
   - ✓ Chemokine mediated transendothelial migration.

6- Firm adhesion to tissue matrix proteins
   - ✓ CD44 on T-cells with hyaluronan
   - ✓ VLA4 on T-cells with Fibronectin

Humoral Immunity

Definition:

- It is the arm of the adaptive immune response that is mediated by secreted antibodies "in Blood & lumen of mucosal organs".

- It is important for protection against extracellular microbes & microbial toxins.

- ***The Type and amount of antibodies vary according to:***
  - ✓ The type of Antigen
  - ✓ Involvement of T-cells
  - ✓ Prior history of Antigen Exposure
  - ✓ The anatomic site at which activation occurs

- Multivalent non-protein Antigens with **repeating determinants** such as: **polysaccharides, some lipids and nucleic acids** are able to elicit strong B-cell response through ***Cross Linkage*** of BCRs especially if associated with strong complement activation. This response is accomplished without the need for T-Cell help so called **T-Independent Response**, and the Antigen is called **T-Independent Antigen**.

- On the contrary, protein antigens require T-cell help. The Antigen are internalized by specific B cells, processed and the peptides presented to CD4 helper T–cells **"Follicular Helper T-cells-T_{FH}** "accordingly this response is called **T-dependent** and characterized by strong Antibody production with **Isotype Switching** and **Affinity maturation**.

- Different subsets of B cells respond preferentially to proteins and non-protein Antigens:
  1. **Follicular B-cells "recirculating B-cells":** circulate in the blood, reside in the follicles of the 2^{ry} lymphoid organs and migrate from one secondary lymphoid organ to another searchingfor antigen. These cells mount **T-dependent Antibody response.**
  2. **Marginal B-cells in the spleen & B_1** cells in mucosa "in mucosal associated lymphoid tissues" and peritoneum mount **T-independent Antibody response.**

❖ **About Antibodies?**

- ✓ ***Emil von Behring*** and ***Shibasaburo Kitasato*** in 1890 settled the first experimental proof of humoral immunity. They showed that if serum transferred from animals that had been immunized with attenuated Diphteria toxin, was transferred to naïve animals, those recipient became resistant to diphtheria toxin, so the active component in the serum that offer protection is called Antitoxin.
- ✓ ***Paul Ehrichin 1890***s postulated that immune cells use receptors calling then side chains to recognize microbial toxins and secrete them to combat microbes, also gave them the term ***Antikorper" In German"= Antibodies.***
- ✓ Plasma or serum proteins can be physically separated based on solubility into albumins & globulins and further separation by migration in an electric field "electrophoresis". The antibodies are found in the third latest migratory group of globulin named gamma globulin, so another common name for antibodies is ***immunoglobulin.***
- ✓ ***Almroth Wright*** postulated in the early 1900 that factors in immune serum enhanced the phagocytosis of bacteria by coating, a process known as ***Opsonization.***
- ✓ A healthy 70 kg adult person produces about 2-3 gm of antibodies/day. Two-thirds of them are IgA.

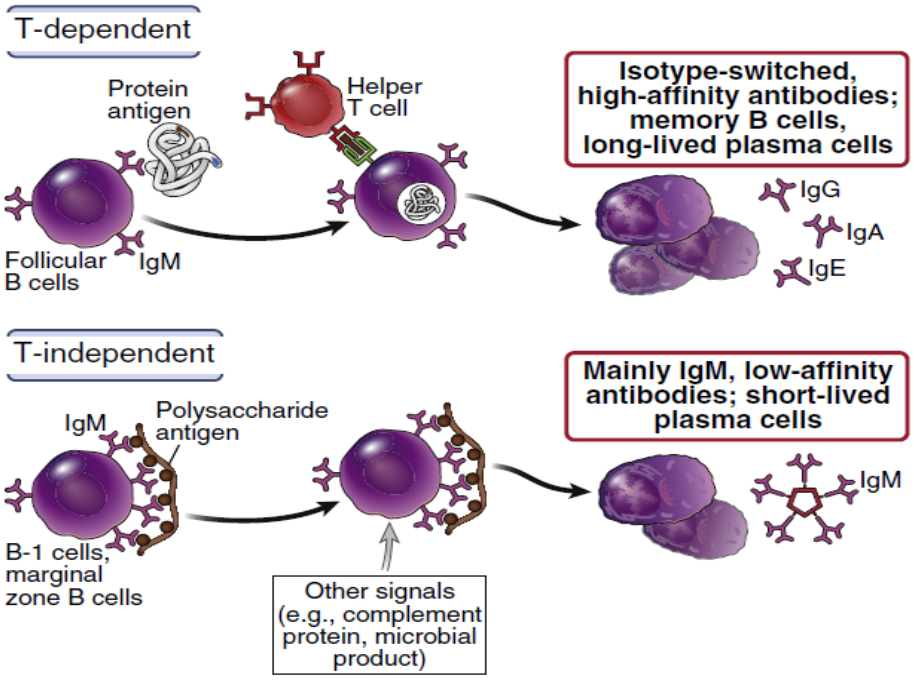


FIGURE 7-2 **T-dependent and T-independent antibody responses.** Antibody responses to protein antigens require T cell help, and the antibodies produced typically show isotype switching and are of high affinity. Non-protein (e.g., polysaccharide) antigens are able to activate B cells without T cell help. Most T-dependent responses are made by follicular B cells, whereas marginal zone B cells and B-1 cells play greater roles in T-independent responses. *Ig,* Immunoglobulin.

T-dependent response:

- Antigen recognition & B-cell activation:

The antigen that is presented to B-cells is in its intact native conformation and is not processed by APCs

- Tissue Antigens may be delivered to lymphoid follicles through:
  1. Afferent lymphatic vessels that drain into subcapsular sinus of the nodes. The small "soluble" antigens may reach through conduits extending between the sinus & the follicles. Larger antigens reach via subcapsular macrophages or resident dendritic cells.
  2. Antigens in Immune complex may bind to CR2 on :
     -*Marginal zone B-cells in spleen* → Antigen-specific Follicular B-cell
     -*Follicular dendritic cell* → Antigen- specific follicular B-cell

- Blood- born antigen may be captured by plasmacystoid dendritic cell in blood and transported to the spleen.

NB: Survival of follicular B-cells depends on:
BAFF: "B cell activating factor of the TNF family" which is one of TNF superfamily cytokine also called **BLyS** = B lymphocyte stimulation. Its ligand on B cell is called APRIL which can activate two other receptors: ***TACI & BCMA***

- Interaction between Antigen and the Antigen –specific B cells through its receptor→ ***Signal 1***" The Antigen is endocytosed, processed and presented to T_{FH}"
- For full activation and subsequent proliferation and differentiation **Second Signal** is needed which may be offered from:
  -The complement fragment C3d "attached to the Antigen" binding CR2 "CD21" on B-cells
  -Microbial products engaging TLR on B cells.

❖ Functional responses of B cell activation:

1- Production of antiapoptotic proteins "Bcl-2"
2- Increased expression of class II MHC molecules and B7 costimulators.
3- Expression of cytokines receptors
4- Change of chemokine receptor expression
5- Increased proliferation and differentiation into:
   - **Effector cells**: "short lived plasma cells": secreting antibodies.
   - **Memory cells**: don't secrete antibodies

✓ Can be further divided into:

a. Effector memory in mucosal tissues, can give rise to rapid Ab on encountering Antigen
b. Central memory cells reside in bone marrow

- **<u>Long lived plasma cells:</u>** in bone marrow, continue to secrete antibodies for years.

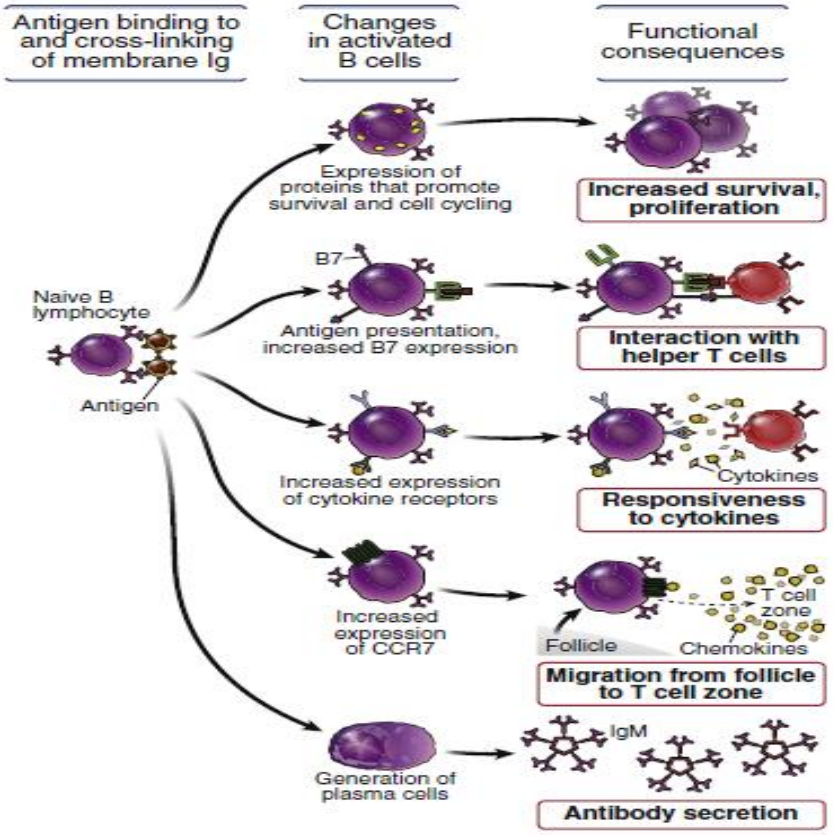


FIGURE 7-6 Functional consequences of antigen receptor-mediated B cell activation. The activation of B cells by antigen in lymphoid organs initiates the process of B cell proliferation and IgM secretion and prepares the B cell for interaction with helper T cells.

❖ <u>Sequence of T-cell help:</u>

- The activation of specific B and T cells by the ***same antigen*** is essential for their functional interaction.
- Following Antigen recognition both T and B cells reverse the expression of chemokine receptors that is :

  -B-cell respond by ↓expression of CXCR5 and ↑expression of CCR7 and T-cell respond by ↓expression of ***CCR7*** and ↑expression of ***CXCR5***.

  -The net result that both antigen activated T and B cells move towards each other.
- At the edge of the follicule "extra-follicular region", B-cell processes the Antigen into different epitopes and presenting these epitopes to Ag-specific T helper cell.

-Binding through CD40L & CD40→proliferation and differentiation into ***PLASMA CELLS*** with weak Antibody response and some degree of isotype switching but of low affinity.

- Some activated B-cells migrate back to follicles accompanied by helper T cells that further activated by B-lymphocyte to develop into follicular helper T cells "T_{FH}" expressing:
  - ✓ *ICOS:* belong to CD28 family interact with ICOSL on B-cells
  - ✓ Bcl6 Transcription factor→ proliferation and anti-apoptotic effect
  - ✓ *CXCR5*
  - ✓ *PD-1*
  - ✓ *IL-21*→secretion which is needed for germinal center development and generation of plasma cells

In addition to IL-21 T_{FH} also secretes IFN-γ, IL-4 or IL-17

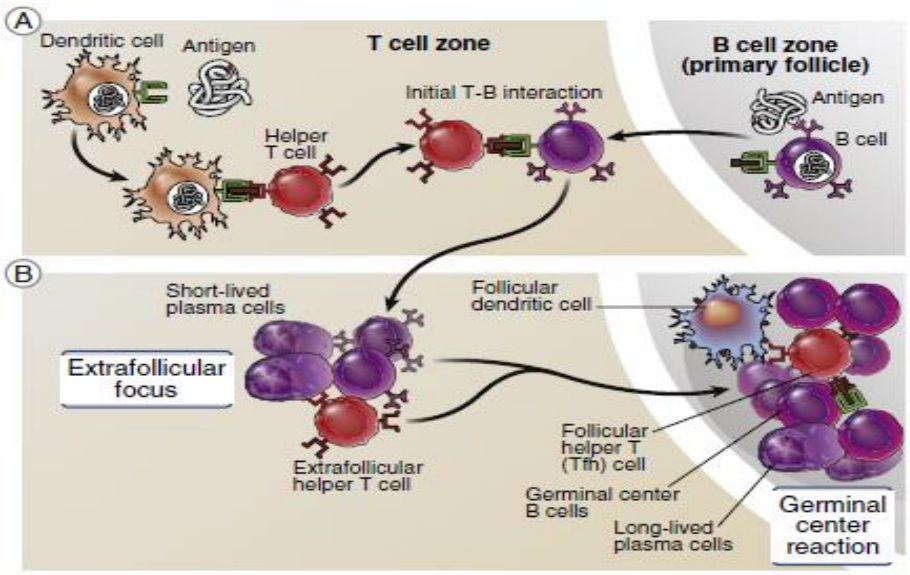


FIGURE 7-7 Sequence of events in helper T cell–dependent antibody responses. **A,** T and B lymphocytes independently recognize the antigen in different regions of peripheral lymphoid organs and are activated. The activated cells migrate toward one another and interact at the edges of lymphoid follicles. **B,** Antibody-secreting plasma cells are initially produced in the extrafollicular focus where the antigen-activated T and B cells interact. Some of the activated B and T cells migrate back into the follicle to form the germinal center, where the antibody response develops fully.

- In the germinal centers the proliferating B cells undergo ***Somatic Hypermutation*** of Ab genes of variable regions and Ig **heavy chain isotype switching**. The highest affinity B-cells are selected for differentiation into long lived plasma cells and memory cells.

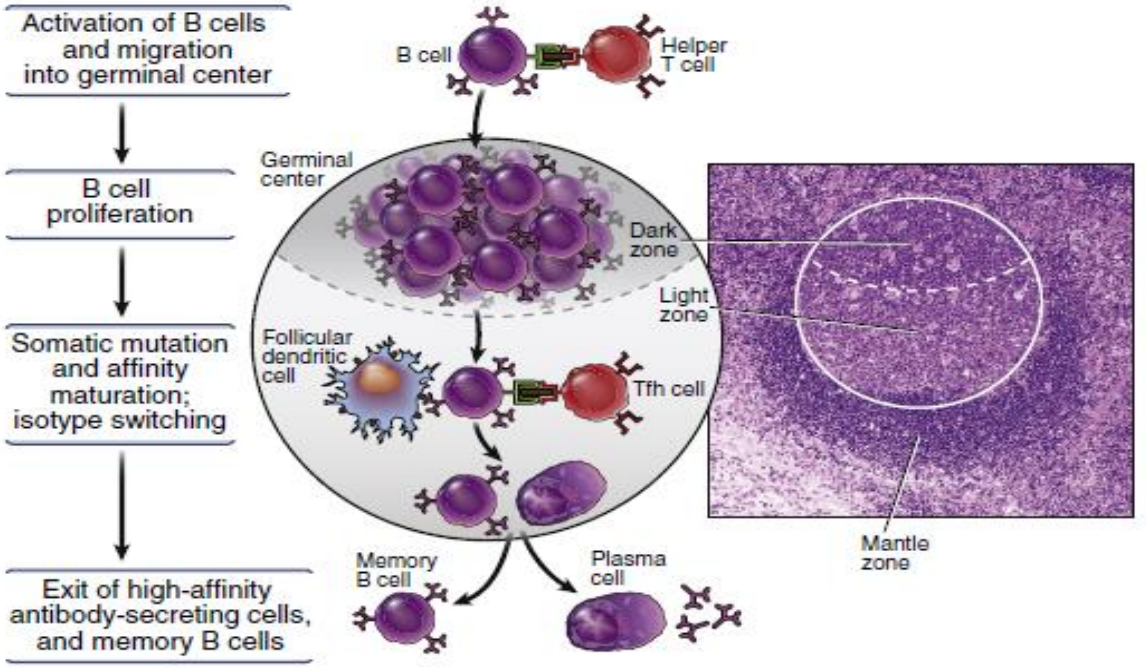


FIGURE 7-10 The germinal center reaction. B cells that have been activated by T helper cells at the edge of a primary follicle migrate into the follicle and proliferate, forming the dark zone of the germinal center. Germinal center B cells undergo extensive isotype switching and somatic mutation of Ig genes, and migrate into the light zone, where B cells with the highest affinity Ig receptors are selected to survive, and they differentiate into plasma cells or memory cells, which leave the germinal center. The right panel shows the histology of a secondary follicle with a germinal center in a lymph node. The germinal center includes a basal dark zone and an adjacent light zone. The mantle zone is the part of the follicle outside the germinal center.

❖ Isotype Switching:

- ✓ Different isotypes= Different functions

- **IgM:** -Complement activation
- **IgG $_1$&G$_3$:**-Opsonization and phagocytosis
  -Complement activation
  -Neonatal immunity
- **IgE, IgG$_4$:** -Eosinophils activation
  -Mast cell degranulation
- **IgA:** -Mucosal immunity

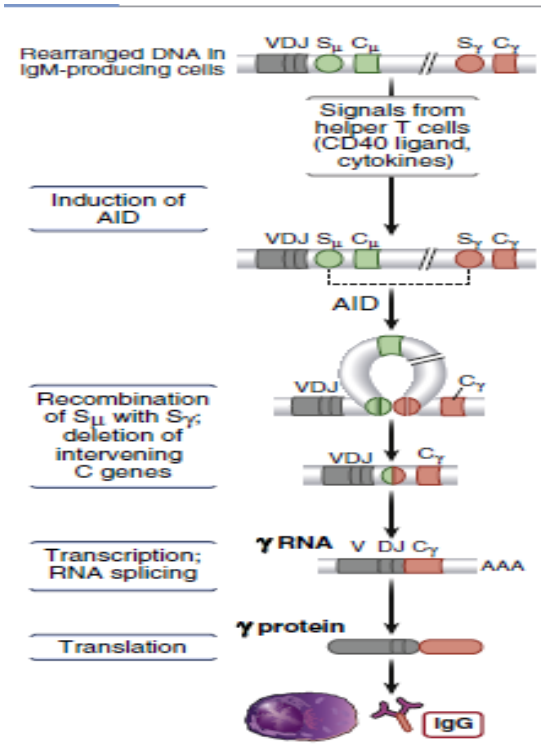
- **Mechanism**:

-Switch recombination: The Ig heavy chain DNA is cut and recombined such that a previously formed VDJ exon that encodes the V region is placed adjacent to another C heavy chain locus.

-This is induced by CD40l-CD40 interaction that leads to expression of the enzyme activation induced deaminase **(AID)**.

-The **cytokines** produced and the anatomic site of activation will determine the isotype subclass:

1. IFN-γ→ IgG
2. IL-4→ IgE
3. TGF-β& mucosal tissue→ Ig A



Mechanism of immunoglobulin heavy-chain isotype switching. In an IgM-producing B cell, the rearranged VDJ heavy-chain gene is adjacent to the μ constant region genes (Cμ). Signals from helper T cells (CD40 ligand and cytokines) may induce recombination of switch (S) regions such that the rearranged VDJ DNA is moved close to a C gene downstream of Cμ, which are Cγ genes in the example shown. The enzyme activation-induced deaminase (AID), which is induced in the B cells by signals from Tfh cells, alters nucleotides in the switch regions so that they can be cleaved by other enzymes and joined to downstream switch regions. Subsequently, when the heavy chain gene is transcribed, the VDJ exon is spliced onto the exons of the downstream C gene, producing a heavy chain with a new constant region and thus a new class of Ig. Note that although the C region changes, the VDJ region, and thus the specificity of the antibody, is preserved. (Each C region gene consists of multiple exons, but only one is shown for simplicity.)

❖ Affinity Maturation:

-It is the process by which the affinity of antibodies produced to a protein antigen increases with prolonged or repeated exposure to that antigen.

- **Mechanism:**

1. Somatic hypermutation in the V region genes in dividing B-cells "In the germinal centers of lymphoid follicles" particularly in the hypervariable regions by the aid of **(AID)** → generation of B-cell clones that bind with varying affinities to the Ag that initiated the response.
2. Ag-Ab complexes are formed and bind to Fc receptors on the surface of FDC to be presented to different B cell clones.
3. As the immune response develops the amount of Ab to that Ag increases especially with repeated exposure and the amount of Ag↓.

4. So the B cells that are selected to survive are those able to bind the Ag at lower and lower concentrations i.e. their BCRs are of higher and higher affinity.

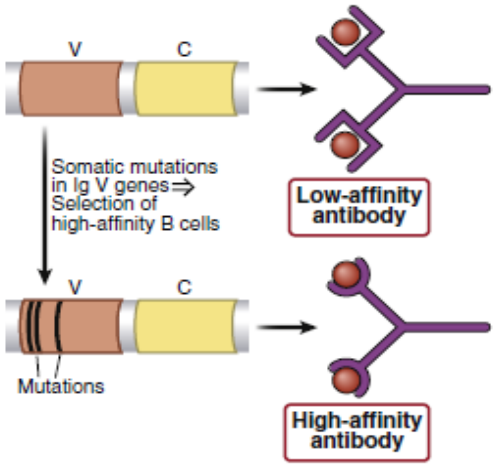


FIGURE 7-13 Affinity maturation in antibody responses. Early in the immune response, low-affinity antibodies are produced. During the germinal center reaction, somatic mutation of Ig V genes and selection of mutated B cells with high-affinity antigen receptors result in the production of antibodies with high affinity for antigen.

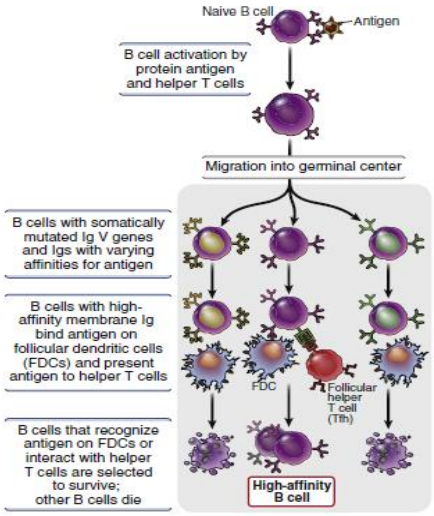


FIGURE 7-14 Selection of high-affinity B cells in germinal centers. Some activated B cells migrate into follicles to form germinal centers, where they undergo rapid proliferation and accumulate mutations in their immunoglobulin (Ig) V genes. These B cells produce antibodies with different affinities for the antigen. Follicular dendritic cells (FDCs) display the antigen, and B cells that recognize the antigen are selected to survive. FDCs display antigens by utilizing Fc receptors to bind immune complexes or by using C3 receptors to bind immune complexes with attached C3b and C3d complement proteins (not shown). B cells also bind the antigen, process it, and present it to follicular helper T (Tfh) cells in the germinal centers, and signals from the Tfh cells promote survival of the B cells. As more antibody is produced, the amount of available antigen decreases, so only the B cells that express receptors with higher affinities can bind the antigen and are selected to survive.

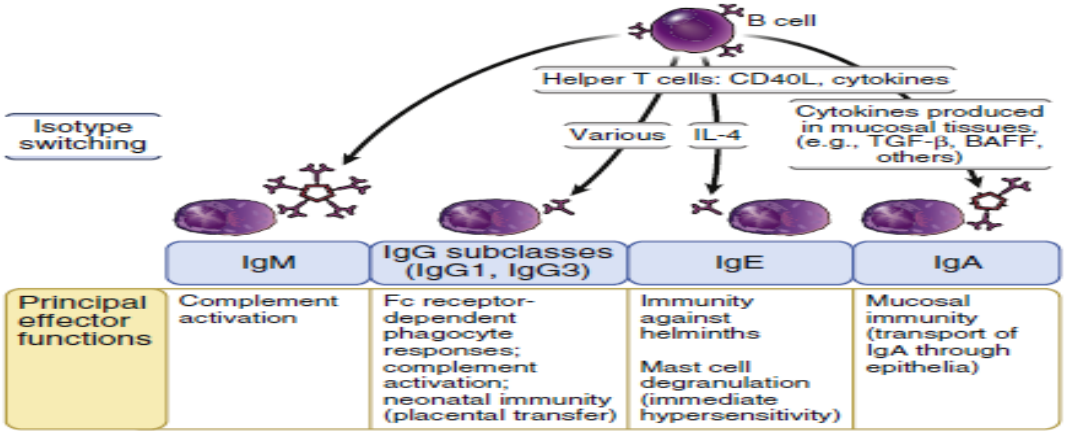


FIGURE 7-11 Immunoglobulin (Ig) heavy-chain isotype (class) switching. Antigen-stimulated B lymphocytes may differentiate into IgM antibody-secreting cells, or, under the influence of CD40 ligand (CD40L) and cytokines, some of the B cells may differentiate into cells that produce different Ig heavy-chain isotypes. The principal effector functions of some of these isotypes are listed; all isotypes may function to neutralize microbes and toxins. BAFF is a B cell–activating cytokine that may be involved in switching to IgA, especially in T-independent responses. Switching to IgG subclasses is stimulated by the cytokine interferon (IFN)-γ in mice, but in humans it is thought to be stimulated by other cytokines. *IL-4*, Interleukin-4; *TGF-β*, transforming growth factor β.

○ T-independent response:

- *Type of antigen:*

-Non-protein "Polysaccharides, Glycolipids, Nucleic acid"

-*Characterized by*: repeated identical antigenic epitopes "***Multivalent"***→ cross linking

-In addition can induce strong complement activation→ generating C3d fragment→ Signal 2

- B-cells subsets:

  -Marginal zone B-cells

  -B_1 cells.

- The response may show little isotype switching "IgG_2" which may be mediated by cytokines "BAFF & APRIL produced from macrophages and dendritic cells. These cytokines can induced the enzyme (AID).

- The protection via this response is especially important for:
  1. Encapsulated bacteria rich in polysaccharides in their cell wall like pneumococcus, meningeococcus and Haemophilus.
  2. Generation of natural antibodies e.g. from B_1 cells stimulated by bacteria that colonize the GIT.

❖ Regulation of humoral immune responses by Fc receptors: **Antibody feedback:**

-mediated by binding Fc portion of IgG to FcγR II B expressed on B cells→ inhibitory signals shut off BCR induced signals.

-This receptor is also expressed on macrophages &dendritic cells→ inhibition of innate immune responses.

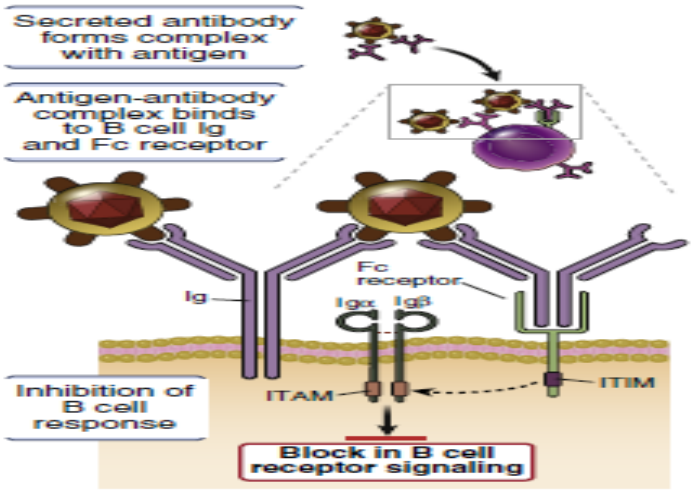


FIGURE 7-16 Mechanism of antibody feedback. Secreted IgG antibodies form immune complexes (antigen-antibody complexes) with residual antigen (shown here as a virus but more commonly is a soluble antigen). The complexes interact with B cells specific for the antigen, with the membrane immunoglobulin (Ig) antigen receptors recognizing epitopes of the antigen and a certain type of Fc receptor (FcγRIIB) recognizing the bound antibody. The Fc receptors block activating signals from the antigen receptor, terminating B cell activation. The cytoplasmic domain of B cell FcγRIIB contains an ITIM that binds enzymes that inhibit antigen receptor–mediated B cell activation. *ITAM*, Immunoreceptor tyrosine-based activation motif; *ITIM*, immunoreceptor tyrosine-based inhibition motif.

❖ ***Effector mechanisms of Humoral Immunity:***

1. Neutralization.
2. Opsonization and phagocytosis.
3. Antibody dependent cellular cytotoxicity (ADCC)
4. Complement activation.

- **Neutralization:**

-Binding of the antibodies by their Fab portions to the microbes on microbial toxins neutralize their infectivity and injurious effects through preventing them from binding tissue cells thus control spread of infection.

-Any isotype can do this mechanism, however the most neutralizing antibodies in blood are of IgG isotype and in mucosa are IgA isotype.

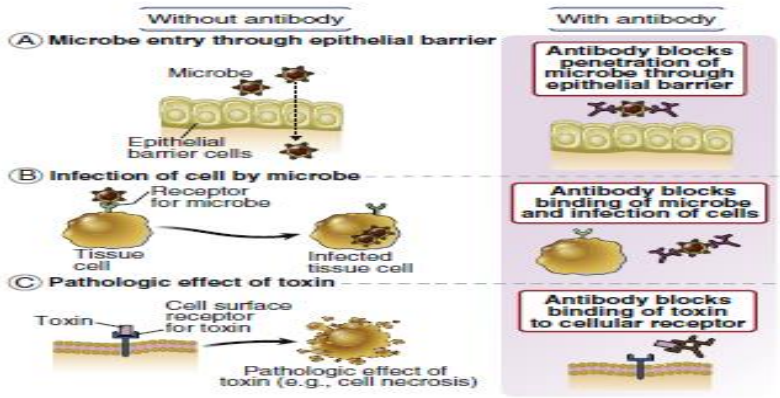


FIGURE 8-4 Neutralization of microbes and toxins by antibodies. **A,** Antibodies at epithelial surfaces, such as in the gastrointestinal and respiratory tracts, block the entry of ingested and inhaled microbes, respectively. **B,** Antibodies prevent the binding of microbes to cells, thereby blocking the ability of the microbes to infect host cells. **C,** Antibodies block the binding of toxins to cells, thereby inhibiting the pathologic effects of the toxins.

- **Opsonization & Phagocytosis:**

-Antibodies of igG isotype can "opsonize" microbes and promote their pathogenesis by binding to Fc receptors on phagocytes particularly FcRI (CD64) which bind Fc portion of IgG_1& IgG_3 with high affinity. FcγR II A, FcγRII C & FcRγII B are low affinity receptors and may be involved in phagocytosis.

-Other Fc receptors for IgG or other isotypes mediate different effector functions.

e.g. FcRγII B→ Antibody feedback "inhibition"

FcRγIII A (CD16) → ADCC

FcƐRI→ Degranulation of mast cells FcγRn" neonatal FcR"→ recycling of IgG→ prolong the half-life of the IgG Ab to weeks.

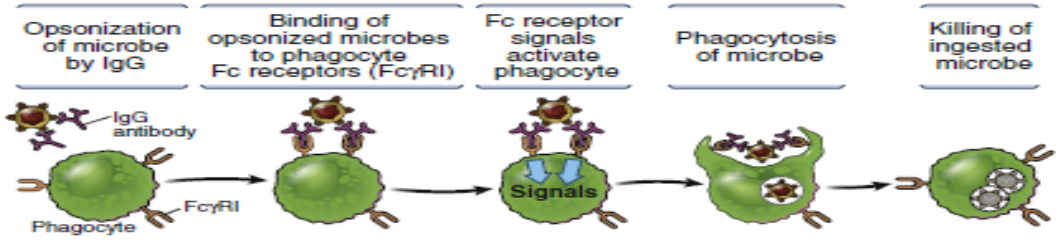


FIGURE 8-5 Antibody-mediated opsonization and phagocytosis of microbes. Antibodies of certain IgG subclasses bind to microbes and are then recognized by Fc receptors on phagocytes. Signals from the Fc receptors promote the phagocytosis of the opsonized microbes and activate the phagocytes to destroy these microbes.

- **<u>Antibody dependent cell mediated cytotoxicity (ADCC):</u>**

  -Occurs when antibodies "IgG" coat microbes on the surface of infected cells, through their Fab fragments. Then through their Fc portions they bind FcRyIII A on NK cells→ NK cells activation→ killing of infected cells.

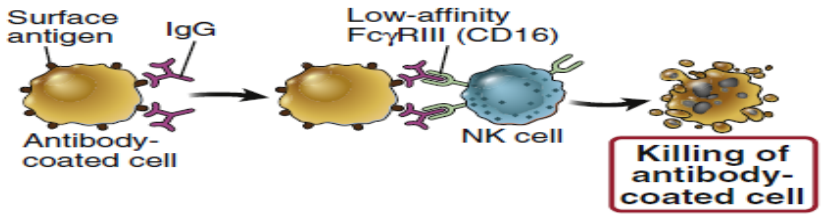


FIGURE 8-7 Antibody-dependent cellular cytotoxicity (ADCC). Antibodies of certain immunoglobulin G (IgG) subclasses (IgG1 and IgG3) bind to cells (e.g., infected cells), and their Fc regions are recognized by an Fcγ receptor on natural killer (NK) cells. The NK cells are activated and kill the antibody-coated cells.

- **<u>Complement activation:</u>**

  - *<u>"About Nomenclature":</u>*

-Jukes Bordet demonstrated that when serum containing an antimicrobial antibody is added to the bacteria at room temperature→ bacterial lysis. If the serum is heated to 56° C or more→ No lysis.

So, he concluded that as the Abs are relatively heat stable the serum must contain another heat-labile component that assists or ***complements*** the lytic function of the antibodies.

-In relation to complement activation, the effector arm of humoral immune response is accomplished through the classical activation pathway of complement that initiates after binding of antibodies with microbes.

- *Functional "effector" sequences of complement activation:*

1. *Opsonization and phagocytosis:*
   -mediated by complement fragments especially C3b & C4b that act as opsonins coating the microbe and facilitate its phagocytosis through complement receptors expressed on phagocytic cells "mainly CR1"
2. *Stimulation of inflammatory response*
   -C3a,C4a & C5a "***chemo-attractants"→*** recruit neutrophils to site of infection
   → They also stimulate degranulation of mast cells→ histamine release
3. *Complement mediated pathogen lysis*
4. *Solubilzation of immune complexes and their clearance by phagocytosis*
   -mediated mainly by binding to CR1 receptors on erythrocytes→ transport of IC to the liver &spleen where phagocytes remove ICs from RBCs & RBCs continue to circulate.
5. *C3d Fragment offers Signal 2 to B cells by binding to CR2 receptors→ B-cell activation*
6. *Opsonized antigens by complement fragment as displayed on the surface of follicular dendritic cells →* ***Affinity Maturation***

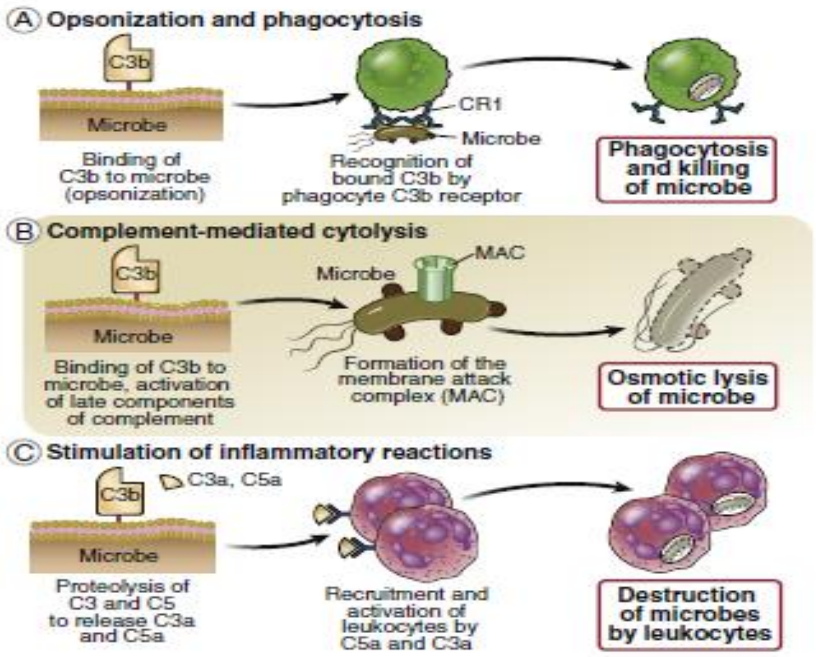


FIGURE 8-11 The functions of complement. **A,** C3b opsonizes microbes and is recognized by the type 1 complement receptor (CR1) of phagocytes, resulting in ingestion and intracellular killing of the opsonized microbes. Thus, C3b is an opsonin. CR1 also recognizes C4b, which may serve the same function. Other complement products, such as the inactivated form of C3b (iC3b), also bind to microbes and are recognized by other receptors on phagocytes (e.g., type 3 complement receptor, a member of integrin family of proteins). **B,** Membrane attack complex creates pores in cell membranes and induces osmotic lysis of the cells. **C,** Small peptides released during complement activation bind to receptors on neutrophils and other leukocytes and stimulate inflammatory reactions. The peptides that

- ***Pathologic effects of complement system:***

  -In spite of the action of inhibitory molecules that regulate or limit complement activation in blood & tissue, complement activation may result in ***tissue damage***

  -Mechanism:

  1. Exaggerated acute inflammatory response
  2. Intravascular thrombosis→ ischemic tissue injury
     ? *Mechanism→ Antiendothelial antibodies "in some autoimmune diseases & organ transplant rejection"→ bind to vascular endothelium& activate complement →*
     MAC→ Damage to endothelial surface→ ***favors Coagulation*** *& also late complement component → ++ prothrombinases→* ***Thrombosis.***
  3. Defective clearance of ICs & their deposition in tissues e.g. Kidneys→ immune complex disease e.g. vasculitis, SLE nephritis.

Clinical significance of complement measurement

- Complement increases as part of the acute phase response.
- Reduction of serum complement occurs in any disease associated with circulating immune complexes or autoantibodies of the IgM or IgG class.
- Measurement of serum complement may be useful in the following situations:
  - ✓ Conditions featuring immune complexes such as SLE, Sjogren syndrome, mixed cryoglobulinemia, serum sickness and some forms of vasculitis and glomerulonephritis.
  - ✓ Cold agglutinins in which C3 fragments are present on the surface of RBCs and occasionally cause hemolytic anemia.
  - ✓ Recurrent infections particularly encapsulated organisms; neisserial infections being the most common.

Human leukocyte Antigens

MHC vs HLA

- *Major histocompatibility complex **(MHC):***
  -Large collection of genes that were originally defined to be responsible for determining the acceptance or rejection of tissue grafts.
  -Later on shown to have a fundamental importance of all immune responses to protein antigens.
  -Their products are membrane bound proteins expressed on the cell surfaces.

- *Human Leukocyte Antigens **(HLA):***
  -Term for Human MHC gene products expressed on the surface of human cells

- *Why Leukocyte?* :
  -An appellation that derives from recognition in the 1950s that many people particularly those who had received multiple blood transfusions and multiparas women had Abs in their serum that reacted with leukocytes.

Genetic terminology & historical background:

- ***Locus:*** The segment of chromosome that contains a single gene.
- ***Alleles:*** Alternative forms of genes.
- ***Region:*** The general portion of the chromosome that contains two or more genes with related origin or function.
- ***Haplotype:*** group of genes inherited as a unit.
- ***Genotype:*** The various genes under consideration in an individual
- ***Phenotype:*** The expression of the genotype as gene products and their resultant interaction.
- ***Polymorphism:*** the availability in the gene pool of the population of many different allelic forms of a gene at a particular locus.
- ***Syngenic:*** pure strain members in this strain are genetically "homozygous"
  "Syngenic grafts are not rejected"
- ***Allogenic:*** the members express different alleles= genetically not identical "heterozygous".
  "Allogenic grafts are rejected"
- ***Congenic:*** identical for all genes except the one for which they were selected to differ.

- ***Linkage disequilibrium:*** some alleles are found linked together more frequently than could be expected by chance.
  E.g. B8-DR3, DQ2
  B44-DR4, DQ7

- Early studies on animals revealed that transplanted organs from allogenic members did not survive because of a destructive process termed "rejection" takes place 1 or 2 weeks of implantation, whereas the same transplants accepted between syngenic members. These results showed that inherited genes must be involved in the process of tissue rejection.

- By breeding congenic strains the investigations showed that a single genetic region is primarily responsible for the rejection and this region was called the major histocompatibility region (histo=tissue).

- In mice it was linked to a gene on chromosome 17 encoding a blood antigen called **Antigen II** and therefore this region was called histocompatibility-2 **(H-2)** "originally thought to be a locus of single gene, but proved later to be several different genes which are highly polymorphic & so called **Major histocompatibility complex.**

- This represented a puzzle for immunologist, as transplantation is not a natural phenomenon, so they continue their researches in 1960s & 1970s to find out other functions for this group of genes. They studied the ability of animals of a certain strain to make Abs against synthetic protein Ag. The responsiveness "of making Abs or not" was found to be related to a group of genes called immune response **(Ir)** genes. Ir genes proved to be the MHC genes.

- The formal proof of the phenomenon of MHC restriction come from the experiments of Rolf Zinkerngel and Peter Doherty Published in 1974, which can be considered as the turning points in our understanding of the physiological role of MHC genes in the immune response,& for which they received the 1996 Nobel Prize in Medicine.

- For a response to occur the T lymphocyte is **compelled** to recognize the **Ag** with the MHC molecule is **simultaneously** "Law of MHC restriction".

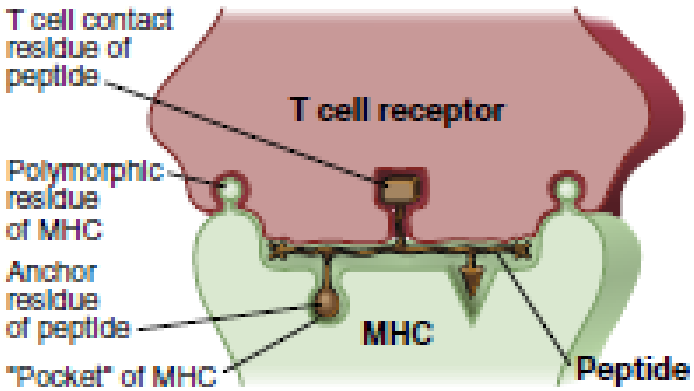


FIGURE 3-1 Model showing how a T cell receptor recognizes a complex of peptide antigen displayed by an MHC molecule.

- The association between the inheritance of particular genes in the MHC and higher risk of developing certain diseases was first clearly demonstrated in **1973** when Brewerton showed that over 90% of patients with AS has B27.

- The first crystallographic identification for structure of class I MHC protein was obtained for HLA.A_2 in 1987 and for class II molecule HLA.DR1 in 1993.

- So the relation between MHC molecules and immune response is definitely existing & evident in:

1. Selection of T-cell clones "repertoire" during development & maturation.
2. Displaying peptide from microbial proteins to Ag specific T-lymphocyte "law of MHC restriction"
3. Differences in a.a sequence of Ag. binding site of MHC molecule→ selective binding of certain epitopes of the protein antigens "determinant selection".

- Owing to the great polymorphism of MHC molecules, so it is not unexpected to have "individuality" of immune response among population. In fact this is evident in:
    2. Particular response to certain epitopes or allergens.
    3. Rejection of allografts.
    4. Susceptibility to develop autoimmune diseases.

MHC genes:

- Present on short arm of chromosome 6 and contain:
  1- Polymorphic MHC genes, Class I & II.
  2- Non -polymorphic genes, Class III→ involve some complement proteins and molecules involved in antigen presentation.
- -Class I & Class II genes are highly polymorphic with >5000 alleles
- Polymorphism ensures that individuals will be able to deal with the **diversity** of microbes and hence protection of population from emerging infections.
- MHC genes are **co-dominantly** expressed in each individual.
- Class I genes lie at the telomeric end, Class II are the most centromeric while Class III lie between I & II.

❖ ***Class I genes:***

-Classical class I genes: named A, B & C encode the α- chain of Class I molecules that are involved in Ag presentation to ***$CD8^+$ lymphocytes.***

-Non classical class I genes: E,F,G some of them encode peptides with important interaction with NK cells.

-In the region in between class I & class III there are:

a. MHC class I related genes "MIC, A and B" encoding molecules of cellular stress
b. TNF-α & HSP.
c. 21-α hydroxylase.

❖ ***Class II genes:***

-DP, DQ, DR encoding molecules that present peptide antigens **to $CD4^+$ lymphocytes.**

- Genes encoding molecules important in antigen loading and processing: DM, DO, TAP genes & proteasome (PSMP).

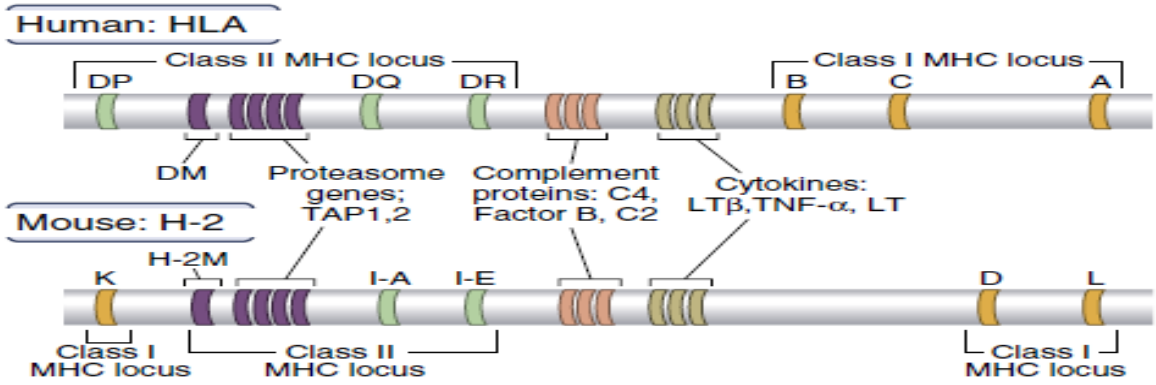
-DR subregion contain a single α chain gene (DRA) i.e. all molecules of the DR subtype have the same chain and more than one (DRB) gene encoding the β –chain of the molecule, the best known is DRB1, An individual may have 2 genes for chain thus expressing 2 DR molecules.

-DQ subregion contains 2 pairs of genes DQA & DQB encoding α and β chains respectively the best characterized is DQA_1& DPB_1 encoding α and β chains of DP molecules.

-DP subregion contains DPA_1 & DPB_1 encoding α and β chains of DP molecules.

❖ ***Class III genes:***

-Encoding complement components.



- **Structure of HLA molecules:**

- ***Class I molecules:*** HLA-A, HLA-B, HLA-C

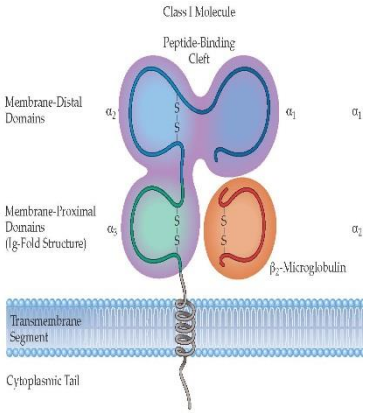
  -formed from polymorphic heavy α chain consists of 3 domains "44KD" binds non-covalently with β chain called B_2 microglobulin "12KD" the gene of which is on chromosome 15.

  -The α-chain is anchored to the cell membrane while the β-chain not.

  -α_1&α_2 domains form the peptide binding groove which accommodates peptides of 8-11 a.a

  .-the floor of the peptide binding groove "cleft" is the site for binding antigen while the sides & top are recognized by the T-cell receptor.

  -**α_3** contain the binding site for T cell co-receptor **CD8.**

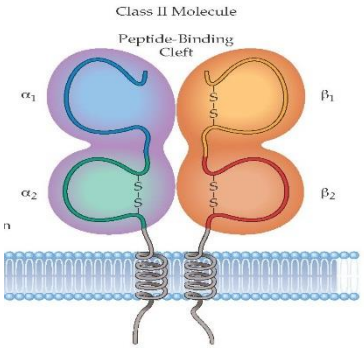


- ***Class II molecules:***

  -consist of 2 chains α and β each chain has 2 domains "α_1- α_2 and β_1-β_2". The carboxy terminal of both chains is anchored to the cell membrane.

  -α_1-and β_1 domains form the peptide binding groove which is larger that of class I and accommodate peptides of 10-30 a.a.

  -β_1 domain contains the binding site for the T-cell coreceptor **CD4.**



Feature	Class I MHC	Class II MHC
Polypeptide chains	a (44–47 kD) b_2-Microglobulin (12 kD)	a (32–34 kD) b (29–32 kD)
Locations of polymorphic residues	a1 and a2 domains	a1 and b1 domains
Binding site for T cell coreceptor	a3 region binds CD8	b2 region binds CD4
Size of peptide-binding cleft	Accommodates peptides of 8-11 residues	Accommodates peptides of 10-30 residues or more
Nomenclature Human	HLA-A, HLA-B, HLA-C	HLA-DR, HLA-DQ, HLA-DP

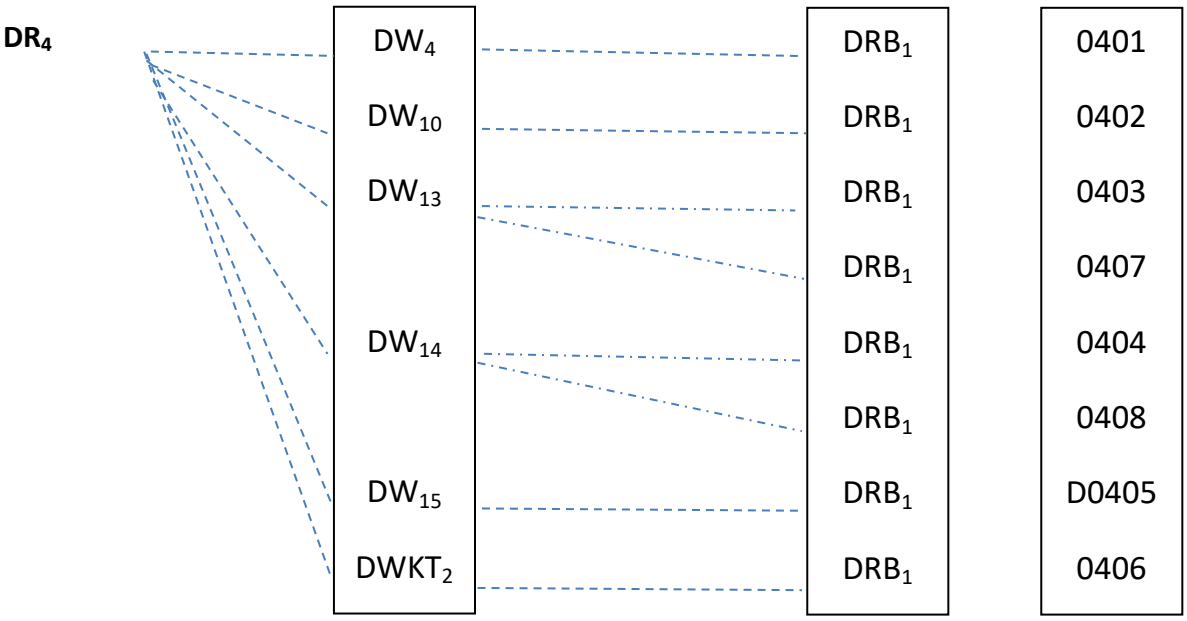
Identification:

Serology → Recognition by multiparons serum or monoclonal antibodies

Cellular typing → Recognition by T-cells in mixed Lymphocyte culture

Oligonucleotide typing → Hybridization of probe with specific DNA sequence

- ***Example:***



-Serology specificity was simply numbered DR_1, DR_2.....etc. Mixed lymphocyte culture (MLC) allowed finer discrimination of allelic differences by cellular recognition by cultured T-lymphocytes of foreign MHC recognition antigen, in this way various subtypes of DR_4 can be identified e.g. DW_4, DW_{10}, DW_{13}, DW_{14} and DW_{15} subtypes of the serologically defined DR_4 specificity. Typing at DNA level or oligonucleotide typing has detailed the fine differences among these alleles.

➢ Nomenclature:

Locus	allele	variant		
DRB_1	04	01	=	DRB_1 0401
DRB	27	02	=	DRB_1 2702

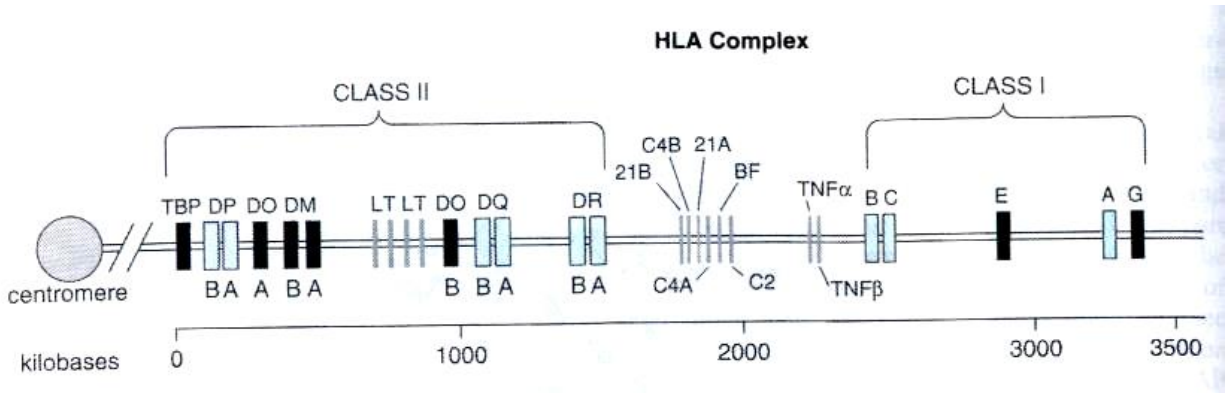
➢ Properties of MHC genes & molecules:

1. Inheritance:

-Highly polymorphic.
-MHC are *co-dominantly* expressed i.e. both genes on the maternal and paternal chromosomes are expressed so an individual may express:

- 6 class I molecules
- 6-8 class II molecules

-Linkage disequilibrium: describes the fact that certain alleles are found together on the same haplotype with great frequency than should occur if recombination during meiosis was random e.g. HALB8 & DR3 found in Caucasian with frequency of 7% i.e. a long segment of chromosome are passed on undisturbed from generation to generation “extended haplotypes”.



2. **Tissue distribution " expression":**

   -**Class I:** on the surface of virtually all cells except mature Erythrocytes & trophoblasts.

   -**Class II:** constitutively on the surface of antigen presenting cells: macrophages, monocytes, dendritic cells & B -lymphocytes.

   N.B: Class II molecules are expressed on thymic epithelial cells & endothelial cells and can be induced on other cell types under the influence of cytokines.

3. **Peptide binding to MHC molecules:**

   -Only peptides no other types of antigens.

   -The floor of the peptide binding groove contains ***pockets*** into which some amino acids of the peptide antigens fit, these a.a. are called ***anchor residues***, the other residues project upwards recognized by TCR.

   -Each MHC molecules presents only one peptide at a time but capable of presenting many different peptides so long they possess the anchor residues that fit in the pockets.

   - MHC molecules acquire their peptide cargo during their biosynthesis, assembly & transport inside cells.

   -Only peptide loaded MHC molecules are stably expressed on cell surfaces and have slow Off-rate and according to the location of antigen the MHC molecule is chosen:

   - Cytosolic antigen→ presented by MHC Class I.
   - Vesicular antigen → presented by MHC Class II.

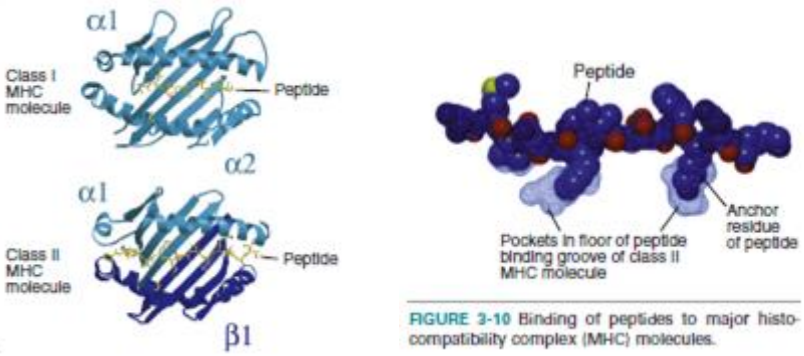
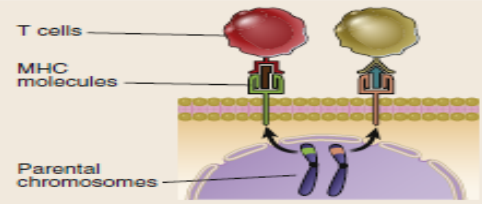


FIGURE 3-10 Binding of peptides to major histocompatibility complex (MHC) molecules.

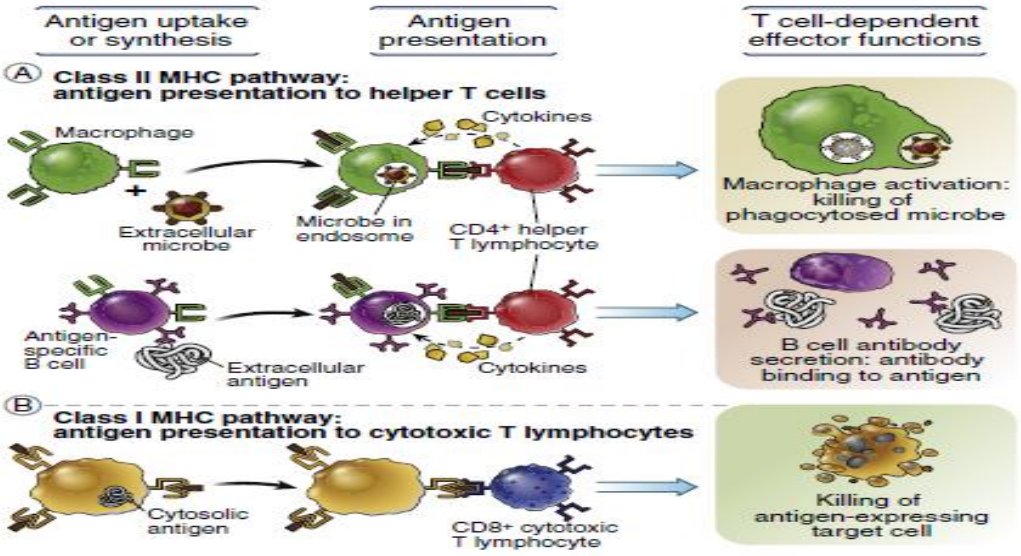
Feature	Significance	
Polymorphic genes: Many different alleles are present in the population	Different individuals are able to present and respond to different microbial peptides	
Co-dominant expression: Both parental alleles of each MHC gene are expressed	Increases number of different MHC molecules that can present peptides to T cells	
MHC-expressing cell types: Class II: Dendritic cells, macrophages, B cells	CD4+ helper T lymphocytes interact with dendritic cells, macrophages, B lymphocytes	
Class I: All nucleated cells	CD8+ CTLs can kill any type of virus-infected cell	

- **<u>The physiological significance of MHC- associated Antigen presentation:</u>**

-The TCRs cannot discriminate between extracellular and intracellular antigens.

-The pathways of antigen processing and presentation are designed to sample all the proteins present in the extracellular & intracellular environment.

-The segregation of antigen processing & presentation by the 2 pathways ensure that the immune system respond differently for these antigens in ways best able to defend against each different microbe or antigen.



-What helps this segregation?

- The site of the antigen inside the cell and the related antigen processing molecules
- The specificity of CD4 molecule to Class II and CD8 molecule to Class I molecules

➢ Relation to Autoimmunity:

-The increased incidence of a particular autoimmune disease with a particular HLA is called Odds ration or RR.

-Possible mechanisms:

1. Certain alleles may be inefficient at displaying certain self-antigens. In the thymus → defective negative selection→ i.e. **emerging of auto reactive T-cells.** "more robbers"
2. Peptide antigens presented by these HLA alleles may fail to stimulate the development of regulatory T-cells i.e **T-reg defects.** "less cops"
3. Certain alleles may be required to present pathogenic self peptides to auto reactive T-cells

Immunologic Tolerance and Autoimmunity

- ***Tolerance= Unresponsiveness***
  Actually it is not absolute. It is controlled so better definition is
  Tolerance = **Controlled** unresponsiveness.

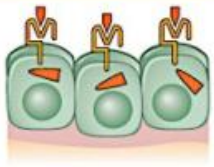
- **Immunological tolerance**: The lack of response to antigens that is induced by exposure of lymphocytes to these antigens.

- When a lymphocyte is exposed to an antigen that can be recognized by its antigen specific receptor (TCR or BCR)

- What is the fate? 1 of 3
  1. Activation, proliferation and differentiation into effector and memory cells .i.e.: generation of immune response. In this case the antigen is said to be **Immunogenic.**
  2. Functional inactivation or killing of lymphocyte. In this case the antigen is said to be **Tolerogenic**
  3. The antigen specific lymphocyte does not react in any way. This has been called **Immunological ignorance.**

- Normally microbes are *Immunogenic* and self-antigens are *Tolerogenic*.

- A single antigen may be ***immunogen*** or ***tolerogen*** depending on whether it is displayed to specific lymphocytes in the presence or absence of ***inflammation and innate immune responses***.

Feature of antigen	Tolerogenic self antigens	Immunogenic foreign antigens
	 Tissue	 Microbe
Location of antigens	Presence in generative organs (some self antigens) induces negative selection and other mechanisms of central tolerance	Presence in blood and peripheral tissues (most microbial antigens) permits concentration in peripheral lymphoid organs
Costimulation	Deficiency of costimulators may lead to T cell anergy or apoptosis, development of Treg, or sensitivity to suppression by Treg	Expression of costimulators, typically seen with microbes, promotes lymphocyte survival and activation
Duration of antigen exposure	Long-lived persistence (throughout life); prolonged TCR engagement may induce anergy and apoptosis	Short exposure to microbial antigen reflects effective immune response

- When do we need tolerance? In other words what are the situations in which tolerance represents an immunological necessity and of great value?
  1- Tolerance to self-antigens→ **to prevent autoimmunity**.
  2- Tolerance to allergen→ **to prevent allergic disease.**
  3- Tolerance to foreign antigens :
     - ✓ In the gut→ **to prevent reactions to food antigens.**
     - ✓ At the end of immune responses→ **to attain homeostasis**
  4- Tolerance in immune privileged tissues including brain, eye, gonads, placenta & fetus **as immune responses and associated inflammation in these parts carry high risk of lethal organ dysfunction & reproduction failure.**

- So induction of Tolerance has been used as a therapeutic strategy in:
  1- Allergy
  2- Autoimmune diseases
  3- Tissue rejection in organ transplantation
  4- Promoting acceptance of stem cell transplants.
  5- Preventing reactions to injected proteins in patients suffering a deficiency of these proteins "hemophilic treated with factor VIII.

Tolerance to self-antigens

- The best studied mechanisms of tolerance are those for CD4+ helper T cells as these cells control virtually all immune responses to protein antigens.
- Therefore if ***helper T cells*** are made unresponsive to self-protein antigens this may be enough to prevent cell mediated and humoral immune responses against self-antigens.

How is a fully armed immune system prevented from self-destruction?

- The process is multi-layered and under the control of various molecules and transcription factors at both central and peripheral levels.
- So a better definition to self-tolerance is the controlled inability to respond to self despite having the capability to do so.

Central T cell tolerance

- The specificities of the TCR encoded by the recombined genes are **random and** are not influenced by what is foreign or self, so it is not surprising to generate lymphocytes with receptors that recognize self-antigens **"auto-reactive".**
- Mechanisms of central tolerance have evolved to prevent the generation of auto-reactive T cells.

- During the process of development & maturation in the thymus, the receptor is forced to engage the environment "surrounding molecules" for 2 main reasons:

1- Test for self-reactivity.
2- Lymphocytes require very low affinity binding to self-antigen to remain viable to complete the process of maturation & also remain viable in the periphery.

The environment "the antigens that are present in the thymus" include:

- ✓ **Serum proteins.**
- ✓ **Cell surface molecules.**
- ✓ **Extracellular matrix proteins.**

Autoimmune regulator protein (AIRE) is responsible for expression of organ – specific extra-thymic molecules "Ags" in the thymus to accomplish the process of negative selection to self-antigens.

- ✓ Mutations in AIRE genes are the cause of a multi-organ autoimmune disease called *autoimmune polyendocrinopathy syndrome type I (APS1)*

The fate of TCRs exposure to self-antigens.

1- Receptors with no affinity: **die by neglect "deleted".**

2- Receptors with high affinity either: **Die by apoptosis "mitochondrial pathway" negatively selected or develop into T regulatory cells (T-reg).**

3- Receptors with low affinity: **Positively selected** to complete the development & maturation process & go to the periphery.

Ignorance: describes a situation of certain antigens not present in the thymus or may be present at insufficient level needed for the process of thymic education: auto reactive cells in the periphery.

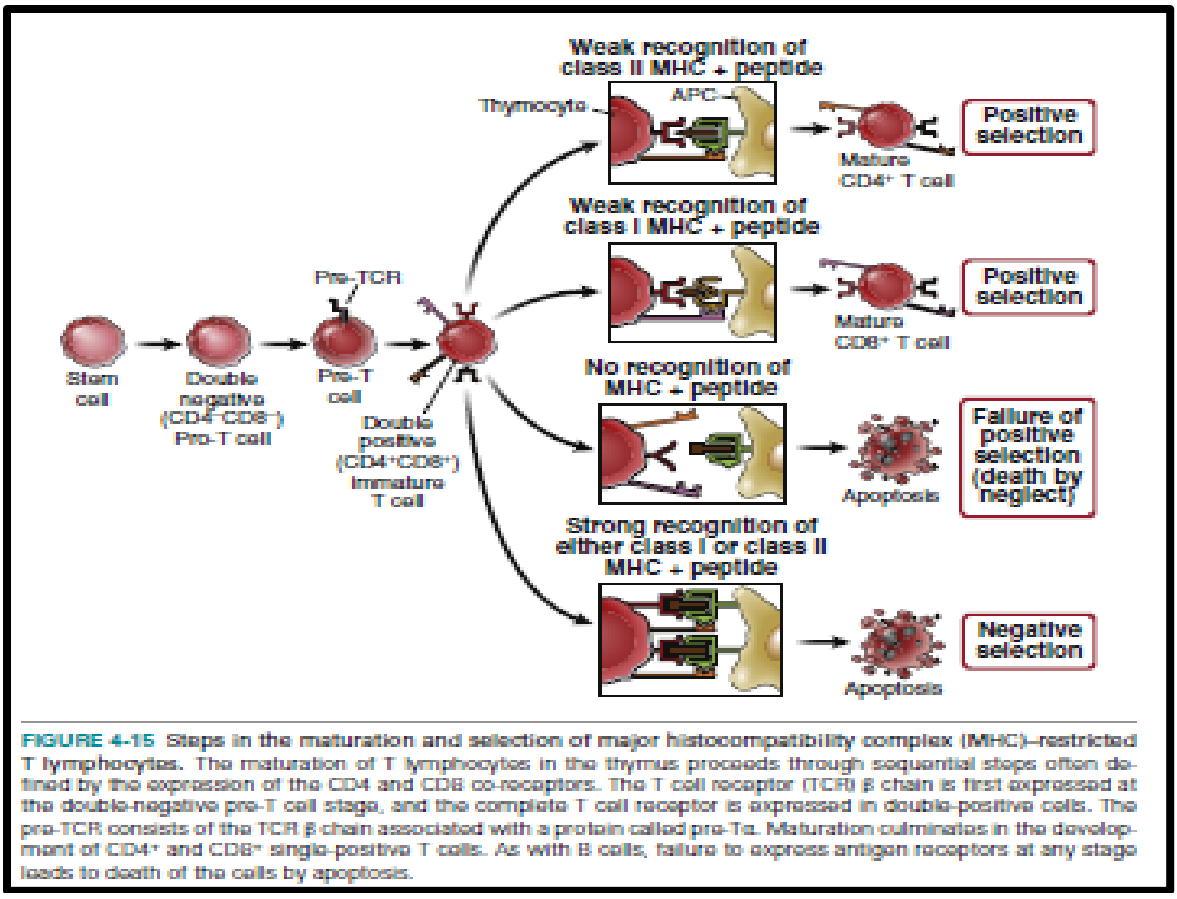
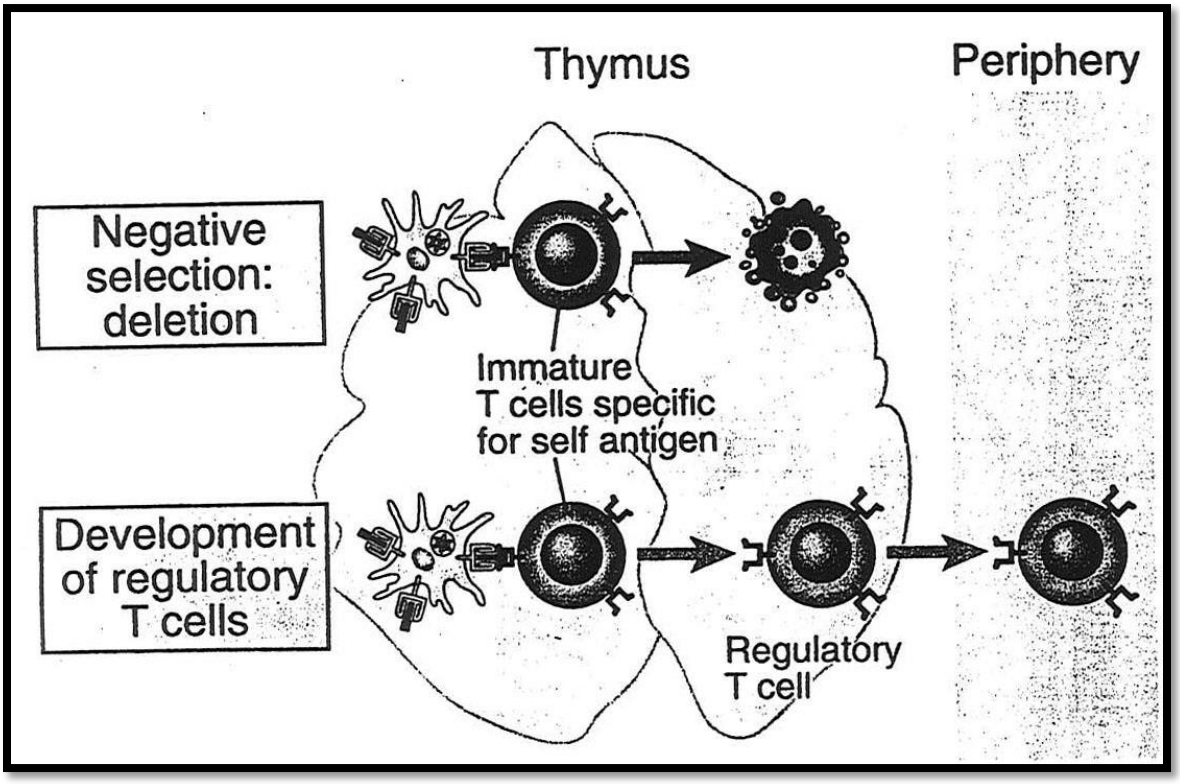


FIGURE 4-15 Steps in the maturation and selection of major histocompatibility complex (MHC)–restricted T lymphocytes. The maturation of T lymphocytes in the thymus proceeds through sequential steps often defined by the expression of the CD4 and CD8 co-receptors. The T cell receptor (TCR) β chain is first expressed at the double-negative pre-T cell stage, and the complete T cell receptor is expressed in double-positive cells. The pre-TCR consists of the TCR β chain associated with a protein called pre-Tα. Maturation culminates in the development of CD4+ and CD8+ single-positive T cells. As with B cells, failure to express antigen receptors at any stage leads to death of the cells by apoptosis.



Peripheral T cell tolerance

- For many reasons central tolerance is incomplete so mechanisms of peripheral tolerance have evolved to prevent unwanted reactions to self-antigens.

Strategies:

- ✓ **Ignore:** mechanisms in peripheral tolerance.
- ✓ **Suppress**: by anergy & T-reg.
- ✓ **Attack**: apoptosis.

A. Ignore

- Immune privilege in the eye, brain & gonads.
- These tissues have evolved to be protected to a variable degree, from immune responses, so as to be protected from lethal organ dysfunction or reproductive failure.

The Eye

- Tight junctions & resistance to leakiness of BVs in tissues adjacent to anterior chamber.
- Absence of lymphatic drainage in anterior chamber.
- Avascular nature of cornea.
- Soluble factors with anti-inflammatory/immunosuppressive junctions: TGFB, neuropeptides.
- Cells lining the anterior chamber including the endothelium & the epithelium of the iris constitutively express Fas lignad & PD-1 with resultant death or inactivation of T cells respectively.
- **N.B**: Self-antigens of the eye are isolated from the immune system during T & B cell development so systemic tolerance to these antigens is not induced. The problem appears with eye trauma: **sympathetic ophthalmia.**

The brain

- Limited permeability of BBB.
- Minimal lymphatic drainage.
- Very few dendritic cells.
- Increased threshold of activation of resident macrophages "microglia".
- Absent or low expression of MHC molecules.
- Neuropeptides anti-inflammatory effects.

B. Suppress:

- Anergy
- T-reg

Anergy "functional unresponsiveness"

- Occurs when TCR recognize self-antigen without co-stimulation: absence of signal 2→ Unresponsiveness.
- The expression of co-stimulators requires strong innate immune response as occurs e.g.: during infection.
- So prolonged signal 1 "Ag & TCR" alone may lead to this state of unresponsiveness.
- *The mechanism*: several biochemical pathways cooperate to maintain this unresponsive state:
  1- Recruitment to the TCR complex of inhibitory molecules such as tyrosine phosphatases.
  2- Activation of Ubiquitin ligase→ Ubiquitinate TCR associated protein proteolytic→ degradation of receptors.
  3- Preferential engagement of the inhibitory receptor CTLA-4.

- The inhibitory molecules with best established role in self-tolerance are: CTLA-4 & PD-1.

CTLA-4: cytotoxic T lymphocyte associated antigen 4 (CD 152) considered the breaker for T cell responses

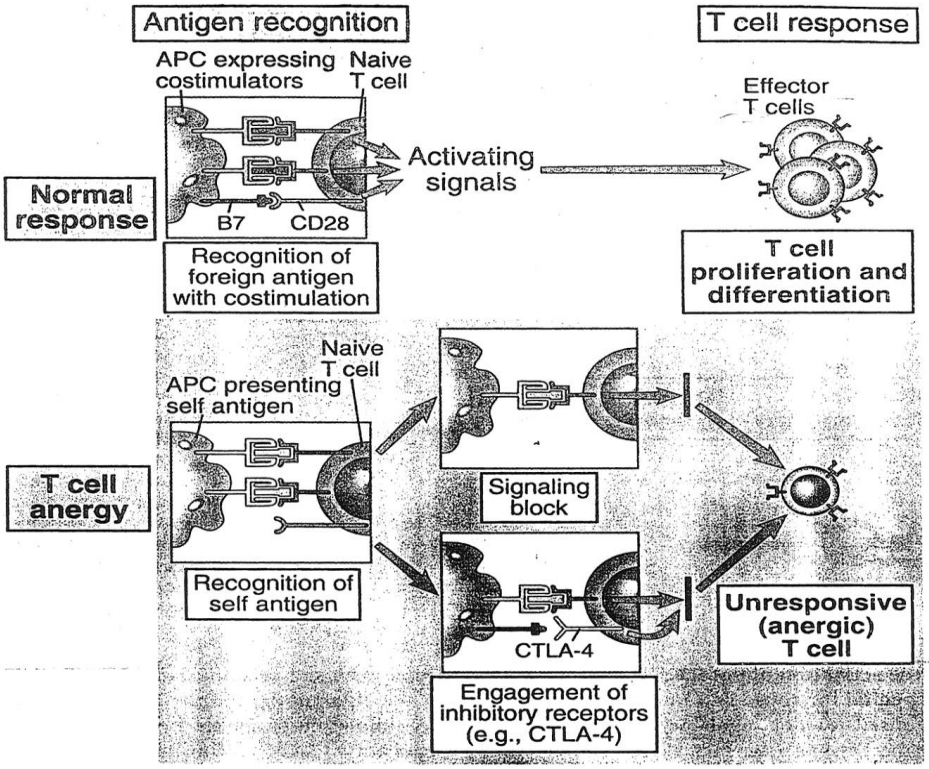
- ✓ Normally its expression on T cells is low & increases after T cell activation. Mediate the inhibitory action via: ***Signaling block*** through activation of phosphatases. Thus when B7 levels are low which is the case when APCs recognize self-antigen, the receptor that is preferentially engaged is the high affinity CTLA-4 with subsequent inhibition "or unresponsiveness" of T cells

- ✓ Monoclonal antibodies blocking CTLA-4 have been used in treatment of tumors .e.g.: melanomas as it restore the normal T cell response against tumors. Some of those treated patients develop autoimmune reactions.

PD-1

- ✓ Clearly important in terminating the peripheral responses of effector T cells especially CD8+.
- ✓ It recognizes 2 ligands PD-L1& PD-L2 expressed on APCS.

N.B.: Other inhibitory receptors have been identified, some belonging to **TNF-receptor family** and other to **TIM family.**



Suppression by regulatory T cells

- T-regs = immunological policemen or cops
- T-regs are a subset of CD4+ cells whose function is to:
    1. Suppress immune responses.
    2. Maintain self-tolerance.

- They may be:
    - ✓ **Natural "central":** develop in thymus by *self-antigens*. Needs FOXP3. Mutation in FOXP3 → IPEX.
    - ✓ **Induced "peripheral"** develop by recognition of *self & foreign Ag* in the peripheral lymphoid organs.

- Absence of strong innate response occurs in some cases with **TGF-β.**
- Particular subset of dendritic cells may be important for development of T-reg, that is dendritic cells exposed to retinoic acid "vit A analogue". This mechanism is especially evident in mucosal lymphoid tissue.
- T-reg cells characterized by high expression of IL-2 receptor α–chain **(CD25)** and transcription factor **FOXP3** in addition to high level of **CTLA-4.**
- The survival & functional competence of T-reg is dependent on IL-2 cytokine which activates STAT5→ Expression of FOXP3.

Mechanisms of action of T-reg

1- Production of anti-inflammatory cytokines IL-10 & TGF-β.

TGF-β: Produced mainly by activated macrophages & CD4+.

- ✓ Decrease proliferation of effector T cells.
- ✓ Decrease activation of **macrophages, neutrophils& endothelial cells.**
- ✓ Decrease Th1 & Th2 subsets development & potentiates Th17 development "**opposing actions".**
- ✓ Increase collagen synthesis & fibrous tissue formation.

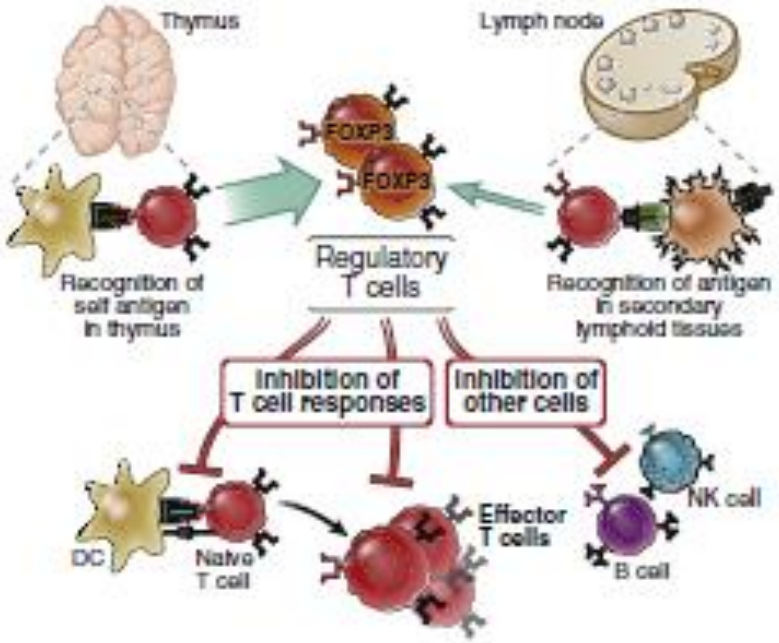
IL-10: Mainly by **macrophages & dendritic cells** and acts to inhibit both cells so functions as **negative feedback regulator.**

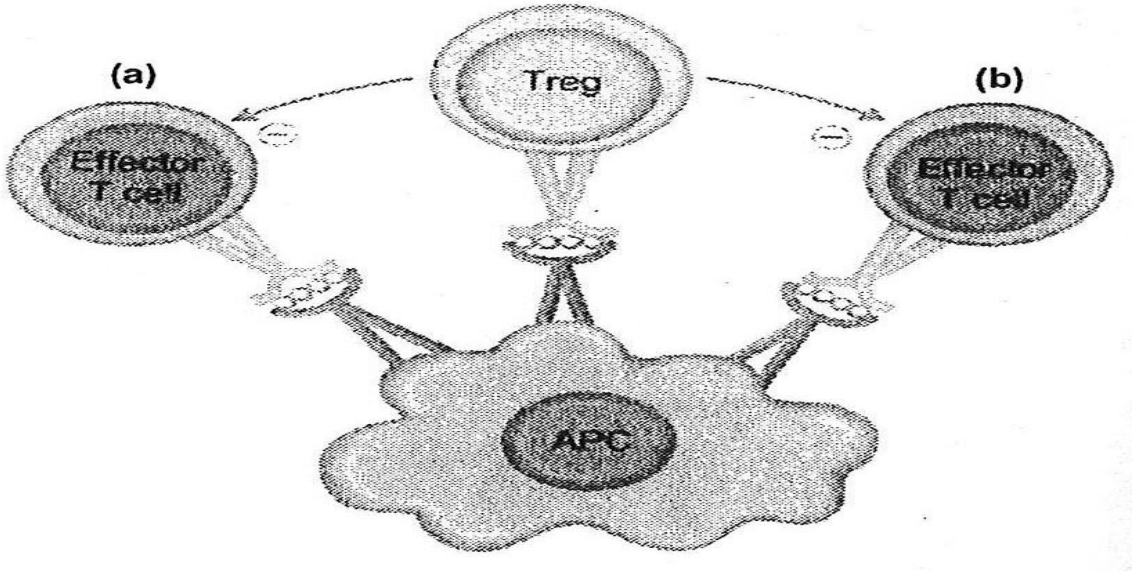
- ✓ Also produced by T-reg & Th1 & Th2.
- ✓ It decrease production of IL-12 so decreases IFN-γ
- ✓ Inhibits expression of Co-stimulators & MHC class II.
- ✓ Mutation of IL-10 receptor: severe colitis that develop early "before 1 year".
- ✓ EBV contains gene homologous to IL-10: inhibits host immunity against virus & prolongs its survival.
- ✓ IL-10 is also produced by some B lymphocytes to which mediate immune suppressive function "regulatory **B cells"**

2- Binding of CTLA-4 on T-reg cells with B7 molecules on dendritic cells so decrease the ability of these cells to stimulate T cells.

3- Consumption of IL-2 as IL-2 receptor is highly expressed on T-regs so deprive other T cells from this important cytokine "considered a growth factor for T cells" so decrease proliferation & differentiation of T cells.

- Defects in T-regs have been linked to various autoimmune diseases & allergic disorders.





T-reg cell recognizing the same self-antigen as auto-reactive T cells & gives inhibitory signals to them.

Therapeutic applications

- ✓ Trials to use T-reg to treat transplant rejections & autoimmune diseases.
- ✓ Induce these cells in autoimmune disease patients by administrating self-peptides that are the targets of autoimmunity either alone or with low doses of IL-2.

C- Attack (Apoptosis)

T lymphocytes that:

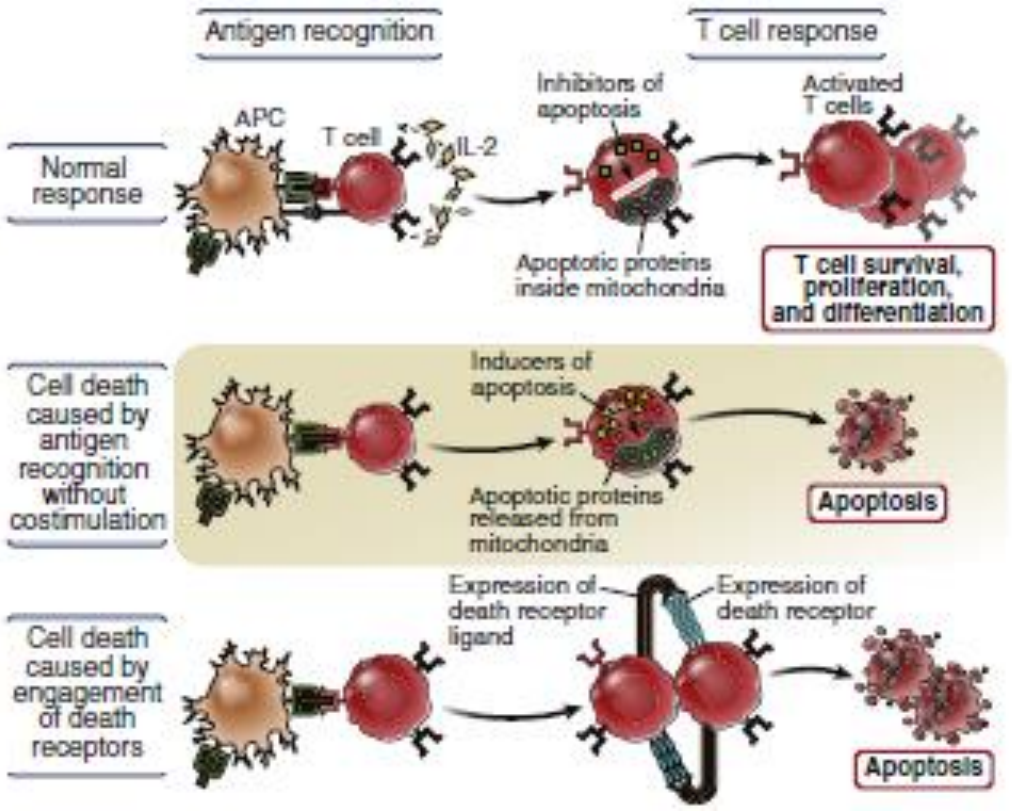
1- Recognize self-antigen with high affinity, or
2- Repeatedly stimulated by antigen.

May die by apoptosis by 2 mechanisms:

1- Antigen recognition induces production of **pro-apoptotic proteins**: death by the mitochondrial pathway through activation of caspases.
2- Antigen recognition leads to co- expression of death receptors and their ligands "**Fas-Fas L ":** apoptosis by death receptor pathway.
Mutations in genes encoding caspases 8 or 10 and Fas signaling → **Autoimmune lymphoproliferative syndrome.**

Peripheral Tolerance of CD8+

1- Inhibitory receptors PD-1.
2- T-reg.
3- High concentration of self Ag: apoptosis.



Peripheral T cell tolerance by apoptosis

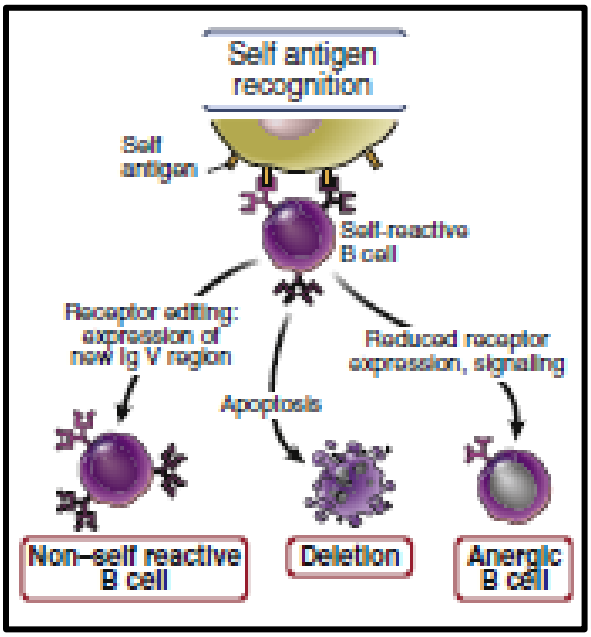
B lymphocyte tolerance

Central tolerance

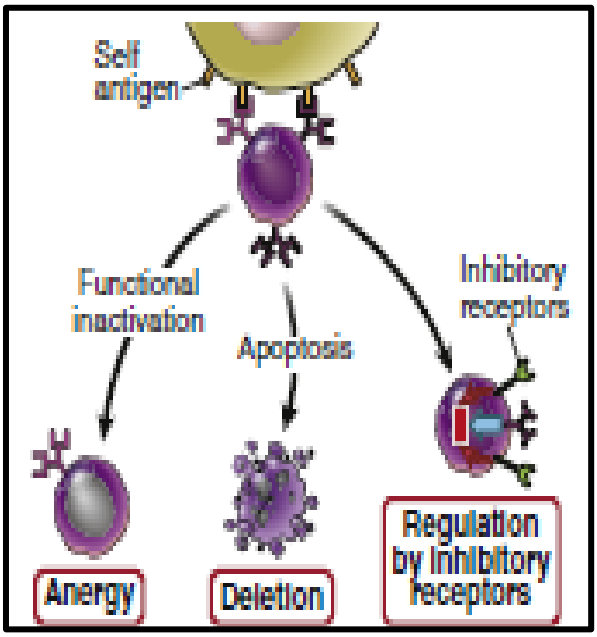
- B cells expressing BCR with high affinity to self-antigen during development → Receptor editing → If failed → Die by apoptosis.
- Developing B cells recognizing soluble self-antigen weakly e.g.: Ag that cannot cross link the receptor may become functionally unresponsive or anergic "block in Ag receptor signaling".

Peripheral tolerance

- Self-antigens are peptides, so B cell response alone may be insufficient: it needs T cell help so without T cell help of the auto reactive B cells: functional unresponsiveness → B cell die by apoptosis.
- Self-reactive B cells that are repeatedly stimulated → unresponsive to further activation due to exhaustion or decrease level of B stimulants BAFF/BLyS. Recognition of self-antigen by auto-reactive B cell may trigger the expression of the inhibitory receptor CD22.



A. Central B tolerance



B. Peripheral B tolerance

Autoimmunity

-In the early 1900s Paul Ehrich coined the rather melodramatic phrase ***horror autotoxicus*** for harmful immune reactions against self.

-Autoimmunity can be defined in a very straightforward way as ***the loss of immunological tolerance to self***.

-Not all auto-reactive T-cells are deleted in the thymus, so all of us have the capacity & potential to mount an autoimmune response. Some of these T-cells recognizing self may be regulatory T-cells i.e. their recognition of self-antigen leads to immune inhibition i.e. physiological autoimmunity. Should the auto reactive T-cells become activated to effectors & cause tissue damage, then autoimmune disease will be the result i.e. the spectrum of autoimmune responses pass from:

Autoimmune potential*→ *Physiological autoimmunity*→ *Pathological autoimmunity

-The key event in developing an autoimmune disease is the **activation of an effector CD4 T-cell that recognizes self-peptide**

-The multilayered nature of self-tolerances is a fail-safe mechanism: all or several control mechanisms must be breached before disease result.

-This approach may explain some features of autoimmune diseases:

1. It is usually multifactorial, requiring genetic predisposition and environmental or other triggers.
2. Often progresses slower than immune reactions to pathogens suggesting that control mechanisms of tolerance continue to work up to a point.
3. Tendency to remissions & exacerbations indicating that control mechanisms may recover & temporarily restore tolerance.
4. Established autoimmune disease tends to be chronic & progressive as many amplification mechanisms may be activated.

-Autoimmune diseases may be either systemic or organ specific depending on the distribution of the auto-antigens that are recognized.

I. Organ specific: Examples
   - Acetylcholine receptors in myasthenia gravis
   - TSH receptors in Grave's disease
   - Cytochrome P450 in autoimmune hepatitis
   - 21α hydroxylase in Addison's disease

II. System or organ non-specific

- IgG in RA
- Various nuclear proteins in SLE
- SS-A & SS-B in Sjogren's syndrome
- Ribonucleoproteins in MCTD

-The principle triad behind the development of autoimmunity is: **Break of tolerance** + **susceptibility gene** + **environmental trigger** e.g. infection or tissue damage

- Immunological abnormalities that lead to autoimmunity may involve the following:
  1. Defective tolerance whether central or peripheral
  2. Abnormal display of Antigen:
     a. Exposure of hidden self-antigen.
     b. Defective clearance
     c. Structural changes of self-antigens from enzymatic modifications or cellular stress or injury
  3. Inflammation or an initial innate immune response:

✓ As occurs during infection or tissue injury.

✓ These may contribute to the development of an autoimmune disease perhaps by ***activating APCs which overcomes regulatory mechanisms and result in excessive T-cell activation***.

- Genetic basis of autoimmunity:
  Most autoimmune diseases are ***complex polygeneic traits*** in which affected individual inherit multiple genetic polymorphisms that contribute to disease susceptibility
- Environmental & other factors: Infection "viral or bacterial" gender, diet, sunlight, cutaneous & intestinal microbiome.

-For activation of a self-reactive CD4 T-cell 4 checkpoint must be overcome:

❖ ***Checkpoint 1: Failure of self-tolerance:***
-Defects in –ve selection at the thymus→ potential of auto reactive T-cells at periphery
-The classic example of central tolerance failure is the defect in AIRE gene→ autoimmune polyglandular syndrome type I (APS) which is inherited as an autosomal dominant disease.

❖ ***Checkpoint 2: Failure of peripheral tolerance:***
-Fewer policemen or ***T-regs*** = more criminals "***auto-reactive T cells***"
-Defects in the development of regulatory T-cells may occur either central or peripheral, in varying degrees may contribute to the development of autoimmune diseases.
-Major defects in T-reg development is seen with X-linked inherited defect in FoXP3 gene→ ***IPEX***= Immune dysregulation, polyendocrinopathy, enteropathy X-linked syndrome.

-Polymorphism of gene that encodes ***CTLA-4*** in human is associated with ↑ risk of developing autoimmune thyroid disease and type I diabetes.

❖ ***Checkpoint 3: Presentation of an auto-antigen "or its mimic"***

a. Tissue damage caused by viral or bacterial infection →release of hidden self-antigens→ processed by tissue resident DC → presented to T-cells in the regional LN.
b. Molecular mimicry: Antigen or epitope in the pathogen looks like Self-antigen.
   Example:
   -Cardiac myocin & streptococcal M-protein → ***Cardiomyopathy in RF***
   - H^+K^+ ATPase "gastric proton pump" & the Helicobacter Pylori acetate Kinase → ***autoimmune gastritis.***

❖ ***Checkpoint 4: Co-stimulation i.e. availability of signal II***

a. There must be sufficiently activated APC in order to offer signal 2 for an auto-reactive T-cell recognizing self-antigen

b. Signal 2 is provided by co-stimulators B7.1 & B7.2 on APC with CD28 on T-cell and may also be provided by pro-inflammatory cytokines e.g. IFN-α has powerful effect similar to that of activated DC. This may explain occurrence of autoimmune reactions with interferon therapy.

c. Usually strong activation of APC occurs in context with infections " viral or bacterial" Infection is associated with:
   1- Expression of <u>co-stimulators</u> on APC and secretions of T-cell activating <u>cytokines.</u>
   2- Local tissue damage with release of auto-antigen.

-Thus the <u>activated APC</u> can present both the ***pathogen*** and ***the self-antigen*** to T-cells in the regional LN with subsequent activation of both ***specific T-cell for pathogen*** and ***specific T-cell for the self Ag***" if present". This is called ***bystander activation.***

- ***Genetic basis of Autoimmunity:***

i. <u>Role of HLA genes</u>:
   -Previously mentioned with HLA.

ii. <u>Role of non-HLA genes</u>:
   -Polymorphism or defects in genes encoding cytokine receptors, complement components, Fc receptors, NOD receptors, autophagic proteins & others may contribute to the development of autoimmune diseases, through:
   - Failure of self-tolerance
   -Abnormal activation of lymphocytes

   Examples:
   - Polymorphism of:
     ✓ PTPN22 "Protein tyrosine phosphatase N22" that regulate B & T cell activation has been associated with RA. SLE & type 1 diabetes.

- ✓ Cytoplasmic sensor NOD2→ reduced resistance to intestinal microbes → Crohn's disease.
- ✓ Gene encoding IL-2 receptor α chain "CD25"→ influence the balance between effector & regulatory T-cells.
- ✓ Receptor for cytokine IL-23→ exaggerated development of proinflammatory Th17.
- ✓ CTLA-4 " previously mentioned
- ✓ ATG16L1: one of the autophagic proteins which is involved in capture of microbes and targets them to lysosomes. Also important in maintenance of epithelial cell integrity, its polymorphism has been associated with ***defective autophagic clearance of intracellular microbes*** & may predispose to inflammatory bowel disease.

Appendix: Cytokines

Cytokine and Subunits	Principal Cell Source	Cytokine Receptor and Subunits*	Principal Cellular Targets and Biologic Effects
Type I Cytokine Family Members			
Interleukin-2 (IL-2)	T cells	CD25 (IL-2Rα) CD122 (IL-2Rβ) CD132 (γc)	T cells: proliferation and differentiation into effector and memory cells; promotes regulatory T cell development, survival, and function NK cells: proliferation, activation
Interleukin-3 (IL-3)	T cells	CD123 (IL-3R) CD131 (βc)	Immature hematopoietic progenitors: induced maturation of all hematopoietic lineages
Interleukin-4 (IL-4)	CD4+ T cells (Th2), mast cells	CD124 (IL-4R) CD132 (γc)	B cells: isotype switching to IgE T cells: Th2 differentiation, proliferation Macrophages: alternative activation and inhibition of IFN-γ–mediated classical activation
Interleukin-5 (IL-5)	CD4+ T cells (Th2), group 2 ILCs	CD125 (IL-5R) CD131 (βc)	Eosinophils: activation, increased generation
Interleukin-6 (IL-6)	Macrophages, endothelial cells, T cells	CD126 (IL-6R) CD130 (gp130)	Liver: synthesis of acute-phase protein B cells: proliferation of antibody-producing cells T cells: Th17 differentiation
Interleukin-7 (IL-7)	Fibroblasts, bone marrow stromal cells	CD127 (IL-7R) CD132 (γc)	Immature lymphoid progenitors: proliferation of early T and B cell progenitors T lymphocytes: survival of naive and memory cells
Interleukin-9 (IL-9)	CD4+ T cells	CD129 (IL-9R) CD132 (γc)	Mast cells, B cells, T cells, and tissue cells: survival and activation
Interleukin-11 (IL-11)	Bone marrow stromal cells	IL-11Rα CD130 (gp130)	Production of platelets
Interleukin-12 (IL-12): IL-12A (p35) IL-12B (p40)	Macrophages, dendritic cells	CD212 (IL-12Rβ1) IL-12Rβ2	T cells: Th1 differentiation NK cells and T cells: IFN-γ synthesis, increased cytotoxic activity
Interleukin-13 (IL-13)	CD4+ T cells (Th2), NKT cells, group 2 ILCs, mast cells	CD213a1 (IL-13Rα1) CD213a2 (IL-13Rα2) CD132 (γc)	B cells: isotype switching to IgE Epithelial cells: increased mucus production Macrophages: alternative activation
Interleukin-15 (IL-15)	Macrophages, other cell types	IL-15Rα CD122 (IL-2Rβ) CD132 (γc)	NK cells: proliferation T cells: survival and proliferation of memory CD8+ cells

Cytokine and Subunits	Principal Cell Source	Cytokine Receptor and Subunits*	Principal Cellular Targets and Biologic Effects
Interleukin-17A (IL-17A) Interleukin-17F (IL-17F)	CD4+ T cells (Th17), group 3 ILCs	CD217 (IL-17RA) IL-17RC	Endothelial cells: increased chemokine production Macrophages: increased chemokine and cytokine production Epithelial cells: GM-CSF and G-CSF production
Interleukin-21 (IL-21)	Th2 cells, Th17cells, Tfh cells	CD360 (IL-21R) CD132 (γc)	B cells: activation, proliferation, differentiation Tfh cells: development Th17 cells: increased generation NK cells: functional maturation
Interleukin-23 (IL-23): IL-23A (p19) IL-12B (p40)	Macrophages, dendritic cells	IL-23R CD212 (IL-12Rβ1)	T cells: differentiation and expansion of Th17 cells
Interleukin-25 (IL-25; IL-17E)	T cells, mast cells, eosinophils, macrophages, mucosal epithelial cells	IL-17RB	T cells and group 2 ILCs: production of IL-5, IL-13
Interleukin-27 (IL-27): IL-27 (p28) EBI3 (IL-27B)	Macrophages, dendritic cells	IL-27Rα CD130 (gp130)	T cells: enhances Th1 differentiation inhibition of Th17 differentiation
Stem cell factor (c-Kit ligand)	Bone marrow stromal cells	CD117 (KIT)	Pluripotent hematopoietic stem cells: induced maturation of all hematopoietic lineages
Granulocyte-monocyte CSF (GM-CSF)	T cells, macrophages, endothelial cells, fibroblasts	CD116 (GM-CSFRα) CD131 (βc)	Immature and committed progenitors: induced maturation of granulocytes and monocytes Macrophage activation
Monocyte CSF (M-CSF, CSF1)	Macrophages, endothelial cells, bone marrow cells, fibroblasts	CD115 (CSF1R)	Committed hematopoietic progenitors: induced maturation of monocytes
Granulocyte CSF (G-CSF, CSF3)	Macrophages, fibroblasts, endothelial cells	CD114 (CSF3R)	Committed hematopoietic progenitors: induced maturation of granulocytes
Type II Cytokine Family Members			
IFN-α (multiple proteins)	Plasmacytoid dendritic cells, macrophages	IFNAR1 CD118 (IFNAR2)	All cells: antiviral state, increased class I MHC expression NK cells: activation
IFN-β	Fibroblasts, plasmacytoid dendritic cells	IFNAR1 CD118 (IFNAR2)	All cells: antiviral state, increased class I MHC expression NK cells: activation
Interferon-γ (IFN-γ)	T cells (Th1, CD8+ T cells), NK cells and group 1 ILCs	CD119 (IFNGR1) IFNGR2	Macrophages: classical activation (increased microbicidal functions) B cells: isotype switching to opsonizing and complement-fixing IgG subclasses (established in mice) T cells: Th1differentiation Various cells: increased expression of class I and class II MHC molecules, increased antigen processing and presentation to T cells

Cytokine and Subunits	Principal Cell Source	Cytokine Receptor and Subunits*	Principal Cellular Targets and Biologic Effects
Interleukin-10 (IL-10)	Macrophages, T cells (mainly regulatory T cells)	CD210 (IL-10Rα) IL-10Rβ	Macrophages, dendritic cells: inhibition of expression of IL-12, costimulators, and class II MHC
Interleukin-22 (IL-22)	Th17 cells	IL-22Rα1 IL-10Rβ2 or IL-22α2 IL-10Rβ2	Epithelial cells: production of defensins, increased barrier function Hepatocytes: survival Adipocytes: lipolysis
Interferon-λs (type III interferons)	Dendritic cells	IFNLR1 (IL-28R) CD210B (IL-10Rβ2)	Epithelial cells: antiviral state
Leukemia inhibitory factor (LIF)	Embryonic trophectoderm Bone marrow stromal cells	CD118 (LIFR) CD130 (gp130)	Stem cells: block in differentiation
Oncostatin M	Bone marrow stromal cells	OSMR CD130 (gp130)	Endothelial cells: regulation of hematopoietic cytokine production
TNF Superfamily Cytokines†			
Tumor necrosis factor (TNF, TNFSF1)	Macrophages, NK cells, T cells	CD120a (TNFRSF1) or CD120b (TNFRSF2)	Endothelial cells: activation (inflammation, coagulation) Neutrophils: activation Hypothalamus: fever Muscle, fat: catabolism (cachexia)
Lymphotoxin-α (LTα, TNFSF1)	T cells, B cells	CD120a (TNFRSF1) or CD120b (TNFRSF2)	Same as TNF
Lymphotoxin-αβ (LTαβ)	T cells, NK cells, follicular B cells, lymphoid inducer cells	LTβR	Lymphoid tissue stromal cells and follicular dendritic cells: chemokine expression and lymphoid organogenesis
BAFF (CD257, TNFSF13B)	Dendritic cells, monocytes, follicular dendritic cells, B cells	BAFF-R (TNFRSF13C) or TACI (TNFRSF13B) or BCMA (TNFRSF17)	B cells: survival, proliferation
APRIL (CD256, TNFSF13)	T cells, dendritic cells, monocytes, follicular dendritic cells	TACI (TNFRSF13B) or BCMA (TNFRSF17)	B cells: survival, proliferation
Osteoprotegrin (OPG, TNFRSF11B)	Osteoblasts	RANKL	Osteoclast precursor cells: inhibits osteoclast differentiation
IL-1 Family Cytokines			
Interleukin-1α (IL-1α)	Macrophages, dendritic cells, fibroblasts, endothelial cells, keratinocytes	CD121a (IL-1R1) IL-1RAP or CD121b (IL-1R2)	Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever Liver: synthesis of acute-phase proteins
Interleukin-1β (IL-1β)	Macrophages, dendritic cells, fibroblasts, endothelial cells, keratinocytes	CD121a (IL-1R1) IL-1RAP or CD121b (IL-1R2)	Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever Liver: synthesis of acute-phase proteins T cells: Th17 differentiation
Interleukin-1 receptor antagonist (IL-1RA)	Macrophages	CD121a (IL-1R1) IL-1RAP	Various cells: competitive antagonist of IL-1

Cytokine and Subunits	Principal Cell Source	Cytokine Receptor and Subunits*	Principal Cellular Targets and Biologic Effects
Interleukin-18 (IL-18)	Monocytes, macrophages, dendritic cells, Kupffer cells, keratinocytes, chondrocytes, synovial fibroblasts, osteoblasts	CD218a (IL-18R) CD218b (IL-18Rβ)	NK cells and T cells: IFN-γ synthesis Monocytes: expression of GM-CSF, TNF, IL-1β Neutrophils: activation, cytokine release
Interleukin-33 (IL-33)	Endothelial cells, smooth muscle cells, keratinocytes, fibroblasts	ST2 (IL1RL1) IL-1 Receptor Accessory Protein (IL1RAP)	T cells: Th2 development ILCs: activation of group 2 ILCs
Other Cytokines			
Transforming growth factor–β (TGF-β)	T cells (mainly Tregs), macrophages, other cell types	TGF-β R1 TGF-β R2 TGF-β R3	T cells: inhibition of proliferation and effector functions; differentiation of Th17 and Treg B cells: inhibition of proliferation; IgA production Macrophages: inhibition of activation; stimulation of angiogenic factors Fibroblasts: increased collagen synthesis

APRIL, A proliferation-inducing ligand; *BAFF*, B cell–activating factor belonging to the TNF family; *BCMA*, B cell maturation protein; *CSF*, colony-stimulating factor; *IFN*, interferon; *ILC*, innate lymphoid cell; *MHC*, major histocompatibility complex; *NK cell*, natural killer cell; *OSMR*, oncostatin M receptor; *RANK*, receptor activator for nuclear factor κB ligand; *RANKL*, RANK ligand; *TACI*, transmembrane activator and calcium modulator and cyclophilin ligand interactor; *TNF*, tumor necrosis factor; *TNFSF*, TNF superfamily; *TNFRSF*, TNF receptor superfamily.

*Most cytokine receptors are dimers or trimers composed of different polypeptide chains, some of which are shared between receptors for different cytokines. The set of polypeptides that compose a functional receptor (cytokine binding plus signaling) for each cytokine are listed. The functions of each subunit polypeptide are not listed.

†All TNF superfamily (TNFSF) members are expressed as cell surface transmembrane proteins, but only the ones that are active as proteolytically released soluble cytokines are listed in the table. Other TNFSF members that function predominantly in the membrane-bound form and are not, strictly speaking, cytokines are not listed in the table. These membrane-bound proteins and the TNFRSF receptors they bind to include OX40L (CD252, TNFSF4):OX40 (CD134, TNFRSF4); CD40L (CD154, TNFSF5):CD40 (TNFRSF5); FasL (CD178, TNFSF6):Fas (CD95, TNFRSF6); CD70 (TNFSF7):CD27 (TNFRSF27); CD153 (TNFSF8):CD30 (TNFRSF8); TRAIL (CD253, TNFSF10):TRAIL-R (TNFRSF10A-D); RANKL (TNFSF11):RANK (TNFRSF11); TWEAK (CD257, TNFSF12):TWEAKR (CD266, TNFRSF12); LIGHT (CD258, TNFSF14):HVEM (TNFRSF14); GITRL (TNFSF18):GITR (TNFRSF18); 4-1BBL:4-1BB (CD137).